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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12N 9/18 // C11D 3/386, C08G 63/91

A1

(11) International Publication Number: **WO 00/34450**

(43) International Publication Date: 15 June 2000 (15.06.00)

(21) International Application Number: PCT/DK99/00678

(22) International Filing Date: 3 December 1999 (03.12.99)

(30) Priority Data:

PA 1998 01604	4 December 1998 (04.12.98)	DK
60/111,591	9 December 1998 (09.12.98)	US
PA 1999 00330	9 March 1999 (09.03.99)	DK
60/124,671	16 March 1999 (16.03.99)	US

(71) Applicant *(for all designated States except US)*: NOVO NORDISK A/S [DK/DK]; Novo Alle, DK–2880 Bagsværd (DK).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: ABO, Masanobu [JP/JP]; 2–5–3, Kouyadai, Funabashi–shi, Chiba–ken 274 (JP). FUKUYAMA, Shiro [JP/JP]; Ariyoshi–cho 166–2, Midori–ku, Chiba–shi, Chiba 211–0012 (JP). SVENDSEN, Allan [DK/DK]; Bakkeledet 28, DK–3460 Birkerød (DK). MATSUI, Tomoko [JP/JP]; Higashi–Ohwada 1–6–9–A303, Ichikawa, Chiba 272–0026 (JP).

(74) Common Representative: NOVO NORDISK A/S; Enzyme Business Patents, Novo Alle, DK–2880 Bagsværd (DK).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: CUTINASE VARIANTS

(57) Abstract

Variants of fungal cutinases have improved thermostability. The variants comprise substitution of one or more amino acid residues near the N–terminal in the amino acid sequence or in the three–dimensional structure of the cutinase.

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CUTINASE VARIANTS

FIELD OF THE INVENTION

The present invention relates to a cutinase variant, more particularly to a cutinase variant having improved thermostability. The invention also relates to a DNA sequence encoding the variant, a vector comprising the DNA sequence, a transformed host cell harboring the DNA sequence or the vector, to a method of producing the variant, and to use of the variant.

BACKGROUND OF THE INVENTION

Cutinases are lipolytic enzymes capable of hydrolyzing the substrate cutin. Cutinases are known from various fungi (P.E. Kolattukudy in "Lipases", Ed. B. Borgström and H.L. Brockman, Elsevier 1984, 471-504). The amino acid sequence and the crystal structure of a cutinase of *Fusarium solani pisi* have been described (S. Longhi et al., Journal of Molecular Biology, 268 (4), 779-799 (1997)). The amino acid sequence of a cutinase from *Humicola insolens* has also been published (US 5,827,719).

A number of variants of the cutinase of *Fusarium solani pisi* have been published: WO 94/14963; WO 94/14964; Appl. Environm. Microbiol. 64, 2794-2799, 1998; Proteins: Structure, Function and Genetics 26, 442-458, 1996; J. of Computational Chemistry 17, 1783-1803, 1996; Protein Engineering 6, 157-165, 1993; Proteins: Structure, Function, and Genetics 33, 253-264, 1998; J. of Biotechnology 66, 11-26, 1998; Biochemistry 35, 398-410, 1996.

Fungal cutinases may be used in the enzymatic hydrolysis of cyclic oligomers of poly(ethylene terephthalate), e.g. in the finishing of yarn or fabric from poly(ethylene terephthalate) fibers (WO 97/27237). However, it is desirable to improve the thermostability of known fungal cutinases to allow a higher process temperature.

SUMMARY OF THE INVENTION

The inventors have found certain variants of fungal cutinases having improved thermostability.

Accordingly, the invention provides a variant of a parent fungal cutinase comprising substitution of one or more amino acid residues which is located:

a) within 17 Å from the location of the N-terminal amino acid (as calculated from amino acid residues in a crystal structure), and/or

b) within 20 positions from the N-terminal amino acid.

The invention also provides a DNA sequence encoding the variant, an expression vector comprising the DNA sequence, a transformed host cell harboring the DNA sequence or the expression vector, a method of producing the variant, processes using the variant and a detergent composition comprising the variant.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 gives the coordinates for the 3D structure of the cutinase of *H. insolens*.

Fig. 2 is a computer model showing the three-dimensional structures of the cutinases from *F. solani pisi* (left) and *H. insolens* (right). Different colors have been used to identify the N-terminal amino acid and zones of 12 Å and 17 Å diameter around this.

Figs. 3-6 illustrate the hydrolysis of c3ET. Details are given in the Examples.

DETAILED DESCRIPTION OF THE INVENTION

Fungal cutinase

The parent cutinase is a fungal cutinase, such as a filamentous fungal cutinase, e.g. native to a strain of *Humicola* or *Fusarium*, specifically *H. insolens* or *F. solani pisi*, more specifically *H. insolens* strain DSM 1800.

The amino acid sequence of the cutinase of *H. insolens* strain DSM 1800 and the DNA sequence encoding it are shown as SEQ ID NO: 2 and SEQ ID NO: 1 of US 5,827,719. The numbering system used herein for the *H. insolens* cutinase is based on the mature peptide, as shown in said SEQ ID NO: 2.

The amino acid sequence of the cutinase of *F. solani pisi* is shown as the mature peptide in Fig. 1D of WO 94/14964. The numbering system used herein for

the *F. solani pisi* cutinase is that used in WO 94/14964; it includes the pro-sequence shown in said Fig. 1D; thus, the mature cutinase is at positions 16-214.

The parent cutinase may have an amino acid sequence which is at least 50 % (particularly at least 70 % or at least 80 %) homologous to the cutinase of *H. insolens* strain DSM 1800. The parent cutinase may particularly be one that can be aligned with the cutinase of *H. insolens* strain DSM 1800.

Nomenclature for amino acids and alterations

The specification and claims refer to amino acids by their one-letter codes. A particular amino acid in a sequence is identified by its one-letter code and its position, e.g. Q1 indicates Gln (glutamine at position 1, i.e. at the N-terminal.

The nomenclature used herein for defining substitutions is basically as described in WO 92/05249. Thus, R51P indicates substitution of R (Arg) at position 51 with P (Pro).

Homology and alignment

For purposes of the present invention, the degree of homology may be suitably determined according to the method described in Needleman, S.B. and Wunsch, C.D., (1970), Journal of Molecular Biology, 48, 443-45, with the following settings for polypeptide sequence comparison: GAP creation penalty of 3.0 and GAP extension penalty of 0.1. The determination may be done by means of a computer program known such as GAP provided in the GCG program package (Program Manual for the Wisconsin Package, Version 8, August 1994, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711).

Two given sequences can be aligned according to the method described in Needleman (*supra*) using the same parameters. This may be done by means of the GAP program (*supra*).

Three-dimensional structure of cutinase

The structure of the cutinase of *H. insolens* was solved in accordance with the principle for X-ray crystallographic methods as given, for example, in X-Ray Structure Determination, Stout, G.K. and Jensen, L.H., John Wiley & Sons, Inc. NY, 1989. The structural coordinates for the solved crystal structure at 2.2 Å resolution

using the isomorphous replacement method are given in Fig. 1 in standard PDB format (Protein Data Bank, Brookhaven National Laboratory, Brookhaven, CT).

The structure of the cutinase of *F. solani pisi* is described in Martinez et al. (1992) Nature 356, 615-618. The 3D structures of the cutinases of *F. solani pisi* and *H. insolens* are compared as a computer model in Fig. 2.

It should be noted that the overall three-dimensional structures of fungal cutinases are very similar and have been shown by X-ray crystallography to be highly homologous. The similarities between the cutinases from *F. solani pisi* and *H. insolens* are clearly apparent from the computer model in Fig. 2. Therefore, modifications of the type indicated for one fungal cutinase will also be functional for other fungal cutinases.

Substitution near N-terminal

The variant of the invention has one or more amino acid substitutions in the vicinity of the N-terminal. The substitution is within a distance of 17 Å (e.g. within 12 Å) and/or within 20 positions (e.g. within 15 positions) of the N-terminal. The distance from the N-terminal is to be calculated between the Cα atom of the amino acids, and is calculated from an amino acid in a crystal structure (i.e. visible in the X-ray structure).

In the cutinase of *H. insolens* strain DSM 1800, the two N-terminal amino acids (Q1 and L2. i.e. Gln and Leu at positions 1 and 2) are not visible in the X-ray structure, so the distance is to be calculated from amino acid G3. Amino acids within 17 Å include positions 3-12, 18, 20-60, 62-64, 82, 85-86, 100-108, 110-111, 130-132, 174, 176-182, 184-185, 188, and 192. Those within 12 Å include positions 3-8, 22-27, 30-47, 53-59, 102, 177, and 180-181.

In the cutinase of *F. solani pisi*, the N-terminal amino acid G17 is visible in the X-ray structure. Amino acids within 17 Å include positions 17-26, 34-75, 77-79, 101, 115, 117-119, 147, 191-197, 199-200, and 203. Those within 12 Å include positions 17-22, 38, 40, 45-58, 60, 65, and 70-72.

The variants of the invention have improved thermostability compared to the parent enzyme. The thermostability may be determined from the denaturation tem-

perature by DSC (differential scanning calorimetry), e.g. as described in an example, e.g. at pH 8.5 with a scan rate of 90 K/hr. The variants may have a denaturation temperature which is at least 5°C higher than the parent enzyme.

The total number of substitutions in the above regions is typically 1-10, e.g. 1-5 substitutions in the above regions. In addition, the cutinase variant of the invention may optionally include other modifications of the parent enzyme, typically 10 or fewer, e.g. 5 or fewer alterations (substitutions, deletions or insertions) outside of the above regions. Thus, the total amino acid sequence of the variant typically 1-20, e.g. 1-10 alterations compared to the parent cutinase.

Solvent accessible surface

One or more of the substitutions may be made at an exposed amino acid residue, i.e. an amino acid residue having a solvent accessible surface. This can be calculated by the "dssp" program (version October 1988) described in W. Kabsch and C. Sander, Biopolymers, 22 (1983) pp. 2577-2637.

In the cutinase of *H. insolens* strain DSM 1800, the following amino acids lie within 17 Å of G3 at the N-terminal and have a solvent accessible surface greater than 0: 3-12, 18, 26-33, 36-38, 40-45, 47-56, 59-60, 62-64, 82, 85-86, 104-105, 174, 176-179, 181-182, 192.

Specific substitutions

The substitution near the N-terminal may specifically be one that increases the electrical charge, i.e. a substitution of a negatively charged amino acid with a neutral or positively charged amino acid or substitution of a neutral amino acid with a positively charged amino acid. Thus, a negative amino acid residue at a position corresponding to position E6, E10, E30, E47 D63, E82 and/or E179 in the cutinase of *Humicola insolens* strain DSM 1800 may be substituted by a neutral or positive amino acid, e.g. R, K, Y, H, Q or N. Some specific substitutions are those corresponding to E6Q/N, E10Q/N, E47K/R or E179Q/N. Also, a neutral amino acid residue at a position corresponding to N7, S11, N44 or N52 in the *H. insolens* cutinase may be substituted by a positive amino acid (R, K or H).

Another example of a substitution near the N-terminal is substitution with a Pro residue, e.g. a substitution corresponding to A14P or R51P in the cutinase of *Humicola insolens* strain DSM 1800.

Specific variants

The following are some examples of variants in the *H. insolens* cutinase. Corresponding variants may be made on the basis of other parent cutinases.

R51P

E6N/Q+ L138I

A14P+ E47K

E47K

E179N/Q

E6N/Q+ E47K+ R51P

A14P+ E47K+ E179N/Q

E47K+ E179N/Q

E47K+ D63N

E6N/Q+ E10N/Q+ A14P+ E47K+ R51P+ E179N/Q

E6N/Q+ A14P+ E47K+ R51P+ E179N/Q

Q1P+ L2V+ S11C+ N15T+ F24Y+ L46I+ E47K

Use of cutinase variant

The cutinase variant of the invention may be used, e.g., for the enzymatic hydrolysis of cyclic oligomers of poly(ethylene terephthalate), such as cyclic tri(ethylene terephthalate), abbreviated as c3ET.

In particular, this may be used to remove such cyclic oligomers from polyester containing fabric or yarn by treating the fabric or yarn with the cutinase variant, optionally followed by rinsing the fabric or yarn with an aqueous solution having a pH in the range of from about pH 7 to about pH 11. The treatment of polyester is conveniently carried out above the glass transition temperature of c3ET (about 55°C) and below the glass transition temperature of polyester (about 70°C). Thus, the treatment may suitably be carried out at 50-80°C, e.g. at 60-75°C. The process may be carried out in analogy with WO 97/27237.

The cutinase variant may be used to treat polyester-containing textile. e.g. PET (polymer of ethyleneglycol and terephthalic acid), P3GT (polymer of 1,3-propanediol and terephthalic acid) or a polyester/cotton blend. The treatment may provide benefits to the polyester textile such as improved wear and comfort, increased water permeability, reduced antistatic behavior, improve handle and softness, changed redeposition characteristics and/or color clarification.

The cutinase variant may be used to improve the functional finish of a PET-containing yarn or fabric by a treatment with the cutinase variant, followed by a treatment with a finishing agent such as a softener, an anti-crease resin, an anti-static agent, an anti-soiling agent or agents to impair wrinkle-free, permanent press ior fire resistance effects. The treatment with the cutinase variant may increase the number of functional groups in the surface, and this can be used to attach the functional finish. Examples of finishing agents are described in "SENSHOKU SIAGEKAKO BENRAN" published 1998-10-15 by Nihon Seni Sentaa KK.

The cutinase variant of the invention is also useful in detergents, where it may be incorporated to improve the removal of fatty soiling, as described in WO 94/03578 and WO 94/14964. The addition of the cutinase variant to laundry detergent may reduce malodor from cloth which is accumulated during several wash/wear-cycles.

The cutinase variant may also be used for degradation and recycling of polyester such as polycaprolactone (PCL), poly-ethyleneglycol-terephthalate (PET), polylactic acid, polybutylenesuccinate, and poly(hydroxybutiric acid)-co-(hydroxyvaleric acid), e.g. film and bottles, e.g. as described in JP-A 5-344897.

The cutinase variant may also be used for other known applications of lipases and cutinases, for example, in the baking industry (e.g. as described in WO 94/04035 and EP 585988), in the papermaking industry (e.g. for pitch removal, see EP 374700), and in the leather, wool and related industries (e.g. for degreasing of animal hides, sheepskin or wool), and for other applications involving degreasing/defatting. It may be used in immobilized form in the fat and oil industry, as a catalyst in organic synthesis (e.g. esterification, transesterification or ester hydrolysis reactions).

Dyeing polyester

The invention provides a process for dyeing polyester fabric or yarn. In this process, the fabric or yarn is first treated with a cutinase, e.g. 12-48 hours at 50-70°C or 65-70°C, pH 7-10, followed by dyeing with dye, e.g. a reactive dye, a disperse dye or a cationic dye. The reactive dye may be one that reacts with OH or COOH groups, e.g. having the structure Chromophore-NHPh-$SO_2CH_2CH_2OSO_3Na$. The dyeing may be conducted at 40-80°C, e.g. for 20-60 minutes.

The cutinase may be a thermostable cutinase having a thermal denaturation temperature, T_d, at pH 8.5 which is at least 5° higher than the parent cutinase, e.g. 7-10° higher, e.g. a value of 65°C or higher. The measurement may be made by DSC as described in an Example of this specification.

Surfactant

In the treatment of fabric or yarn, a conventional wetting agent and/or a dispersing agent may be used to improve the contact with the enzyme. The wetting agent may be a nonionic surfactant, e.g. an ethoxylated fatty alcohol. A very useful wetting agent is an ethoxylated and propoxylated fatty acid ester such as Berol 087 (product of Akzo Nobel, Sweden).

The dispersing agent may suitably be selected from nonionic, anionic, cationic, ampholytic or zwitterionic surfactants. More specifically, the dispersing agent may be selected from carboxymethylcellulose, hydroxypropylcellulose, alkyl aryl sulfonates, long-chain alcohol sulfates (primary and secondary alkyl sulfates), sulfonated olefins, sulfated monoglycerides, sulfated ethers, sulfosuccinates, sulfonated methyl ethers, alkane sulfonates, phosphate esters, alkyl isothionates, acylsarcosides, alkyltaurides, fluorosurfactants, fatty alcohol and alkylphenol condensates, fatty acid condensates, condensates of ethylene oxide with an amine, condensates of ethylene oxide with an amide, sucrose esters, sorbitan esters, alkyloamides, fatty amine oxides, ethoxylated monoamines, ethoxylated diamines, alcohol ethoxylate and mixtures thereof. A very useful dispersing agent is an alcohol ethoxylate such as Berol 08 (product of Akzo Nobel, Sweden).

Methods for preparing cutinase variants

The cutinase variant of the invention can be prepared by methods known in the art, e.g. as described in WO 94/14963 or WO 94/14964 (Unilever). The following describes methods for the cloning of cutinase-encoding DNA sequences, followed by methods for generating mutations at specific sites within the cutinase-encoding sequence.

Cloning a DNA sequence encoding a cutinase

The DNA sequence encoding a parent cutinase may be isolated from any cell or microorganism producing the cutinase in question, using various methods well known in the art. First, a genomic DNA and/or cDNA library should be constructed using chromosomal DNA or messenger RNA from the organism that produces the cutinase to be studied. Then, if the amino acid sequence of the cutinase is known, labeled oligonucleotide probes may be synthesized and used to identify cutinase-encoding clones from a genomic library prepared from the organism in question. Alternatively, a labeled oligonucleotide probe containing sequences homologous to another known cutinase gene could be used as a probe to identify cutinase-encoding clones, using hybridization and washing conditions of lower stringency.

Yet another method for identifying cutinase-encoding clones would involve inserting fragments of genomic DNA into an expression vector, such as a plasmid, transforming cutinase-negative bacteria with the resulting genomic DNA library, and then plating the transformed bacteria onto agar containing a substrate for cutinase (*i.e.* maltose), thereby allowing clones expressing the cutinase to be identified.

Alternatively, the DNA sequence encoding the enzyme may be prepared synthetically by established standard methods, *e.g.* the phosphoroamidite method described S.L. Beaucage and M.H. Caruthers, (1981), Tetrahedron Letters 22, p. 1859-1869, or the method described by Matthes et al., (1984), EMBO J. 3, p. 801-805. In the phosphoroamidite method, oligonucleotides are synthesized, *e.g.* in an automatic DNA synthesizer, purified, annealed, ligated and cloned in appropriate vectors.

Finally, the DNA sequence may be of mixed genomic and synthetic origin, mixed synthetic and cDNA origin or mixed genomic and cDNA origin, prepared by

ligating fragments of synthetic, genomic or cDNA origin (as appropriate, the fragments corresponding to various parts of the entire DNA sequence), in accordance with standard techniques. The DNA sequence may also be prepared by polymerase chain reaction (PCR) using specific primers, for instance as described in US 4,683,202 or R.K. Saiki et al., (1988), Science 239, 1988, pp. 487-491.

Site-directed mutagenesis

Once a cutinase-encoding DNA sequence has been isolated, and desirable sites for mutation identified, mutations may be introduced using synthetic oligonucleotides. These oligonucleotides contain nucleotide sequences flanking the desired mutation sites. In a specific method, a single-stranded gap of DNA, the cutinase-encoding sequence, is created in a vector carrying the cutinase gene. Then the synthetic nucleotide, bearing the desired mutation, is annealed to a homologous portion of the single-stranded DNA. The remaining gap is then filled in with DNA polymerase I (Klenow fragment) and the construct is ligated using T4 ligase. A specific example of this method is described in Morinaga et al., (1984), Biotechnology 2, p. 646-639. US 4,760,025 discloses the introduction of oligonucleotides encoding multiple mutations by performing minor alterations of the cassette. However, an even greater variety of mutations can be introduced at any one time by the Morinaga method, because a multitude of oligonucleotides, of various lengths, can be introduced.

Another method for introducing mutations into cutinase-encoding DNA sequences is described in Nelson and Long, (1989), Analytical Biochemistry 180, p. 147-151. It involves the 3-step generation of a PCR fragment containing the desired mutation introduced by using a chemically synthesized DNA strand as one of the primers in the PCR reactions. From the PCR-generated fragment, a DNA fragment carrying the mutation may be isolated by cleavage with restriction endonucleases and reinserted into an expression plasmid.

Expression of cutinase variants

According to the invention, a DNA sequence encoding the variant produced by methods described above, or by any alternative methods known in the art, can be expressed, in enzyme form, using an expression vector which typically includes con-

trol sequences encoding a promoter, operator, ribosome binding site, translation initiation signal, and, optionally, a repressor gene or various activator genes.

Expression vector

The recombinant expression vector carrying the DNA sequence encoding a cutinase variant of the invention may be any vector which may conveniently be subjected to recombinant DNA procedures, and the choice of vector will often depend on the host cell into which it is to be introduced. The vector may be one which, when introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosome(s) into which it has been integrated. Examples of suitable expression vectors include pMT838.

Promoter

In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell.

Examples of suitable promoters for directing the transcription of the DNA sequence encoding a cutinase variant of the invention, especially in a bacterial host, are the promoter of the *lac* operon of *E.coli*, the *Streptomyces coelicolor* agarase gene *dag*A promoters, the promoters of the *Bacillus licheniformis* α-amylase gene (*amyL*), the promoters of the *Bacillus stearothermophilus* maltogenic amylase gene (*amyM*), the promoters of the *Bacillus amyloliquefaciens* α-amylase (*amyQ*), the promoters of the *Bacillus subtilis* xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding *A. oryzae* TAKA amylase, the TPI (triose phosphate isomerase) promoter from *S. cerevisiae* (Alber et al. (1982), J. Mol. Appl. Genet 1, p. 419-434, *Rhizomucor miehei* aspartic proteinase, *A. niger* neutral α-amylase, *A. niger* acid stable α-amylase, *A. niger* glucoamylase, *Rhizomucor miehei* lipase, *A. oryzae* alkaline protease, *A. oryzae* triose phosphate isomerase or *A. nidulans* acetamidase.

Expression vector

The expression vector of the invention may also comprise a suitable transcription terminator and, in eukaryotes, polyadenylation sequences operably connected to the DNA sequence encoding the α-amylase variant of the invention. Termination and polyadenylation sequences may suitably be derived from the same sources as the promoter.

The vector may further comprise a DNA sequence enabling the vector to replicate in the host cell in question. Examples of such sequences are the origins of replication of plasmids pUC19, pACYC177, pUB110, pE194, pAMB1 and pIJ702.

The vector may also comprise a selectable marker, e.g. a gene the product of which complements a defect in the host cell, such as the *dal* genes from *B. subtilis* or *B. licheniformis*, or one which confers antibiotic resistance such as ampicillin, kanamycin, chloramphenicol or tetracyclin resistance. Furthermore, the vector may comprise *Aspergillus* selection markers such as amdS, argB, niaD and sC, a marker giving rise to hygromycin resistance, or the selection may be accomplished by co-transformation, *e.g.* as described in WO 91/17243.

The procedures used to ligate the DNA construct of the invention encoding a cutinase variant, the promoter, terminator and other elements, respectively, and to insert them into suitable vectors containing the information necessary for replication, are well known to persons skilled in the art (cf., for instance, Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor, 1989).

Host Cells

The cell of the invention, either comprising a DNA construct or an expression vector of the invention as defined above, is advantageously used as a host cell in the recombinant production of a cutinase variant of the invention. The cell may be transformed with the DNA construct of the invention encoding the variant, conveniently by integrating the DNA construct (in one or more copies) in the host chromosome. This integration is generally considered to be an advantage as the DNA sequence is more likely to be stably maintained in the cell. Integration of the DNA constructs into the host chromosome may be performed according to conventional

methods, *e.g.* by homologous or heterologous recombination. Alternatively, the cell may be transformed with an expression vector as described above in connection with the different types of host cells.

The cell of the invention may be a cell of a higher organism such as a mammal or an insect, but is preferably a microbial cell, e.g. a bacterial or a fungal (including yeast) cell.

Examples of suitable bacteria are Gram positive bacteria such as *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus lentus*, *Bacillus brevis*, *Bacillus stearothermophilus*, *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus coagulans*, *Bacillus circulans*, *Bacillus lautus*, *Bacillus megaterium*, *Bacillus thuringiensis*, or *Streptomyces lividans* or *Streptomyces murinus*, or gramnegative bacteria such as *E.coli*. The transformation of the bacteria may, for instance, be effected by protoplast transformation or by using competent cells in a manner known *per se*.

The yeast organism may favorably be selected from a species of *Saccharomyces* or *Schizosaccharomyces*, *e.g. Saccharomyces cerevisiae*.

The host cell may also be a filamentous fungus *e.g.* a strain belonging to a species of *Aspergillus*, most preferably *Aspergillus oryzae* or *Aspergillus niger*, or a strain of *Fusarium*, such as a strain of *Fusarium oxysporium*, *Fusarium graminearum* (in the perfect state named *Gribberella zeae,* previously *Sphaeria zeae, synonym with Gibberella roseum and Gibberella roseum* f. sp. *cerealis)*, or *Fusarium sulphureum (*in the prefect state named *Gibberella puricaris,* synonym with *Fusarium trichothecioides, Fusarium bactridioides, Fusarium sambucium, Fusarium roseum,* and *Fusarium roseum var. graminearum), Fusarium cerealis (*synonym with *Fusarium crokkwellnse*), or *Fusarium venenatum.*

In a preferred embodiment of the invention the host cell is a protease deficient or protease minus strain.

This may for instance be the protease deficient strain *Aspergillus oryzae* JaL 125 having the alkaline protease gene named "alp" deleted. This strain is described in WO 97/35956 (Novo Nordisk).

Filamentous fungi cells may be transformed by a process involving protoplast formation and transformation of the protoplasts followed by regeneration of the cell wall in a manner known per se. The use of *Aspergillus* as a host micro-organism

is described in EP 238 023 (Novo Nordisk A/S), the contents of which are hereby incorporated by reference.

Production of cutinase variant by cultivation of transformant

The invention relates, *inter alia*, to a method of producing a cutinase variant of the invention, which method comprises cultivating a host cell under conditions conducive to the production of the variant and recovering the variant from the cells and/or culture medium.

The medium used to cultivate the cells may be any conventional medium suitable for growing the host cell in question and obtaining expression of the cutinase variant of the invention. Suitable media are available from commercial suppliers or may be prepared according to published recipes (*e.g.* as described in catalogues of the American Type Culture Collection).

The cutinase variant secreted from the host cells may conveniently be recovered from the culture medium by well-known procedures, including separating the cells from the medium by centrifugation or filtration, and precipitating proteinaceous components of the medium by means of a salt such as ammonium sulphate, followed by the use of chromatographic procedures such as ion exchange chromatography, affinity chromatography, or the like.

Expression of variant in plants

The present invention also relates to a transgenic plant, plant part or plant cell which has been transformed with a DNA sequence encoding the variant of the invention so as to express and produce this enzyme in recoverable quantities. The enzyme may be recovered from the plant or plant part. Alternatively, the plant or plant part containing the recombinant enzyme may be used as such.

The transgenic plant can be dicotyledonous or monocotyledonous, for short a dicot or a monocot. Examples of monocot plants are grasses, such as meadow grass (blue grass, Poa), forage grass such as festuca, lolium, temperate grass, such as Agrostis, and cereals, e.g. wheat, oats, rye, barley, rice, sorghum and maize (corn).

Examples of dicot plants are tobacco, legumes, such as lupins, potato, sugar beet, pea, bean and soybean, and cruciferous (family Brassicaceae), such as cauliflower, oil seed rape and the closely related model organism Arabidopsis thaliana.

Examples of plant parts are stem, callus, leaves, root, fruits, seeds, and tubers. In the present context, also specific plant tissues, such as chloroplast, apoplast, mitochondria, vacuole, peroxisomes and cytoplasm are considered to be a plant part. Furthermore, any plant cell, whatever the tissue origin, is considered to be a plant part.

Also included within the scope of the invention are the progeny of such plants, plant parts and plant cells.

The transgenic plant or plant cell expressing the variant of the invention may be constructed in accordance with methods known in the art. In short the plant or plant cell is constructed by incorporating one or more expression constructs encoding the enzyme of the invention into the plant host genome and propagating the resulting modified plant or plant cell into a transgenic plant or plant cell.

Conveniently, the expression construct is a DNA construct which comprises a gene encoding the enzyme of the invention in operable association with appropriate regulatory sequences required for expression of the gene in the plant or plant part of choice. Furthermore, the expression construct may comprise a selectable marker useful for identifying host cells into which the expression construct has been integrated and DNA sequences necessary for introduction of the construct into the plant in question (the latter depends on the DNA introduction method to be used).

The choice of regulatory sequences, such as promoter and terminator sequences and optionally signal or transit sequences is determined, eg on the basis of when, where and how the enzyme is desired to be expressed. For instance, the expression of the gene encoding the enzyme of the invention may be constitutive or inducible, or may be developmental, stage or tissue specific, and the gene product may be targeted to a specific tissue or plant part such as seeds or leaves. Regulatory sequences are eg described by Tague et al, Plant, Phys., 86, 506, 1988.

For constitutive expression the 35S-CaMV promoter may be used (Franck et al., 1980. Cell 21: 285-294). Organ-specific promoters may eg be a promoter from

storage sink tissues such as seeds, potato tubers, and fruits (Edwards & Coruzzi, 1990. Annu. Rev. Genet. 24: 275-303), or from metabolic sink tissues such as meristems (Ito et al., 1994. Plant Mol. Biol. 24: 863-878), a seed specific promoter such as the glutelin, prolamin, globulin or albumin promoter from rice (Wu et al., Plant and Cell Physiology Vol. 39, No. 8 pp. 885-889 (1998)), a *Vicia faba* promoter from the legumin B4 and the unknown seed protein gene from *Vicia faba* described by Conrad U. et al, Journal of Plant Physiology Vol. 152, No. 6 pp. 708-711 (1998), a promotter from a seed oil body protein (Chen et al., Plant and cell physiology vol. 39, No. 9 pp. 935-941 (1998), the storage protein napA promoter from Brassica napus, or any other seed specific promoter known in the art, eg as described in WO 91/14772. Furthermore, the promoter may be a leaf specific promoter such as the rbcs promoter from rice or tomato (Kyozuka et al., Plant Physiology Vol. 102, No. 3 pp. 991-1000 (1993), the chlorella virus adenine methyltransferase gene promoter (Mitra, A. and Higgins, DW, Plant Molecular Biology Vol. 26, No. 1 pp. 85-93 (1994), or the aldP gene promoter from rice (Kagaya et al., Molecular and General Genetics Vol. 248, No. 6 pp. 668-674 (1995), or a wound inducible promoter such as the potato pin2 promoter (Xu et al, Plant Molecular Biology Vol. 22, No. 4 pp. 573-588 (1993).

A promoter enhancer element may be used to achieve higher expression of the enzyme in the plant. For instance, the promoter enhancer element may be an intron which is placed between the promoter and the nucleotide sequence encoding the enzyme. For instance, Xu et al. *op cit* disclose the use of the first intron of the rice actin 1 gene to enhance expression.

The selectable marker gene and any other parts of the expression construct may be chosen from those available in the art.

The DNA construct is incorporated into the plant genome according to conventional techniques known in the art, including *Agrobacterium*-mediated transformation, virus-mediated transformation, micro injection, particle bombardment, biolistic transformation, and electroporation (Gasser et al, Science, 244, 1293; Potrykus, Bio/Techn. 8, 535, 1990; Shimamoto et al, Nature, 338, 274, 1989).

Presently, *Agrobacterium tumefaciens* mediated gene transfer is the method of choice for generating transgenic dicots (for review Hooykas & Schilperoort, 1992.

Plant Mol. Biol. 19: 15-38), however it can also be used for transforming monocots, although other transformation methods are generally preferred for these plants. Presently, the method of choice for generating transgenic monocots is particle bombardment (microscopic gold or tungsten particles coated with the transforming DNA) of embryonic calli or developing embryos (Christou, 1992. Plant J. 2: 275-281; Shimamoto, 1994. Curr. Opin. Biotechnol. 5: 158-162; Vasil et al., 1992. Bio/Technology 10: 667-674). An alternative method for transformation of monocots is based on protoplast transformation as described by Omirulleh S, et al., Plant Molecular biology Vol. 21, No. 3 pp. 415-428 (1993).

Following transformation, the transformants having incorporated the expression construct are selected and regenerated into whole plants according to methods well-known in the art.

MATERIALS AND METHODS

Plasmids

pJSO026

This is a *S. cerevisiae* expression plasmid described in WO 97/07205 and in J.S.Okkels, (1996) "A URA3-promoter deletion in a pYES vector increases the expression level of a fungal lipase in Saccharomyces cerevisiae. Recombinant DNA Biotechnology III: The Integration of Biological and Engineering Sciences, vol. 782 of the Annals of the New York Academy of Sciences).

pFuku83

This is a yeast and E. coli shuttle vector for expression of the H. insolens cutinase under the control of a TPI promoter, constructed from pJSO026.

Substrate

BETEB

Terephthalic acid bis(2-hydroxyethyl)ester dibenzoate is herein abbreviated as BETEB (benzoyl-ethylene-terephthalic-ethelene-benzoate). It was prepared from terephthalic acid bis (2-hydroxyethyl) ester and benzoic acid.

Lipase activity (LU)

A substrate for lipase is prepared by emulsifying tributyrin (glycerin tributyrate) using gum Arabic as emulsifier. The hydrolysis of tributyrin at 30 °C at pH 7 is followed in a pH-stat titration experiment. One unit of lipase activity (1 LU) equals the amount of enzyme capable of releasing 1 µmol butyric acid/min at the standard conditions.

Differential scanning calorimetry (DSC)

Sample and reference solutions are carefully degassed immediately prior to loading of samples into the calorimeter (reference: buffer without enzyme). Sample and reference solutions (approx. 0.5 ml) are thermally pre-equillibrated for 20 minutes at 5°C. The DSC scan is performed from 5 C to 95 C at a scan rate of approx. 90 K/hr. Denaturation temperatures are determined at an accuracy of approx. +/- 1 C. A VP-DSC from MicroCal Inc. is suitable for the experiments.

Methods

PCR conditions

step 1: 94° C, 120 sec.
step 2: 94° C, 60 sec
step 3: 50° C, 60 sec
step 4: 72° C, 150 sec.
Go to step 2, 35 cycles
step 5: 72° C, 480 sec.
Step 6: 4° C, for ever

EXAMPLES

Example 1: Preparation of cutinase variants

A DNA sequence encoding *H. insolens* cutinase was obtained as described in US 5,827,719 (Novo Nordisk) and was found to have the DNA sequence shown in SEQ ID NO: 1 therein.

Variants were prepared by localized random mutagenesis and selection of positive clones by incubation at 60°C for 1 day on BETEB plates. The BETEB plates contained 200 ml/l of 500 mM glycine buffer (pH 8.5), 1.25 g/l of BETEB (dissolved in hot ethanol) and 20 g/l of agar.

Three positive variants were isolated, and their amino acid sequence was determined. They were found to have the following modifications, compared to the parent *H. insolens* cutinase:

A14P + E47K

E47K

E179Q

Example 2: Site directed mutation

A variant of the *H.* insolens cutinase having the substitutions E6Q+ E47K+ R51P was prepared as follows:

A pair of PCR primers were designed so as to introduce amino acid substitutions, making use of the existed restriction enzyme sites nearby, as follows (an asterisk indicates an introduced mutation):

Upper primer: E6Q F

cgg cag ctg gga gcc atc c*ag aac

Pvu II

Lower primer: E47K,R51P

cgc cct gga tcc aga tgt tcg* gga tgt ggg act t*aa ggc

*Bam*H I

PCR was run using these primers and pFukuNL83 as a template under the PCR condition described above.

The obtained PCR fragment was purified by Clontech Spincolumn and digested with *Pvu* II and *Bam*H I.

The resultant fragment was gel-purified and ligated to pFukuNL83 which had been digested with the same restriction enzyme sites.

Example 3: Thermostability of cutinase variants

Variants

The thermostability was tested as described below for the *H. insolens* cutinase and the following variants thereof:

A14P+ E47K

E47K

E179Q

E6Q+ E47K+ R51P

A14P+ E47K+ E179Q

E6Q+ A14P+ E47K+ R51P+ E179Q

E6Q+ E10Q+ A14P+ E47K+ R51P+ E179Q

Differential Scanning Calorimetry (DSC)

Thermostability of cutinase variants was investigated by means of DSC at pH 4.5 (50 mM acetate buffer) and pH 8.5 (50mM glycyl-glycine buffer). The thermal denaturation temperature, T_d, was taken as the top of denaturation peak (major endothermic peak) in thermograms (Cp vs. T) obtained after heating of enzyme solutions at a constant programmed heating rate.

The parent cutinase was found to have T_d of 63°C at pH 8.5. Six of the above variants were found to have T_d of 70-73°C, i.e. an improvement of 7-10°.

The parent cutinase was found to have T_d of 61°C at pH 4.5. Five of the above variants were found to have T_d of 64-66°C, i.e. an improvement of 3-5°.

Hydrolysis of BETEB

The thermostability of the *H. insolens* cutinase and two of the above variants was measured by hydrolysis of BETEB at elevated temperature. For each cutinase, the following mixture was incubated for 17 hours at various temperatures in the range 55-70°C:

0.1 ml 0.5 M glycyl-glycine buffer (pH 8.5)

0.1 ml 0.5 % BETEB dissolved in ethanol

0.1 ml enzyme solution (approx. 25 LU/ml)

0.7 ml Milli Q water

The degree of hydrolysis was measured after the incubation. The results are shown in the table below.

	Variant	Variant	Parent
	27 LU/ml	25 LU/ml	24 LU/ml
55°C	98 %	99 %	72 %
60°C	91 %	83 %	33 %
65°C	66 %	13 %	7 %

These results clearly show that the variants have improved thermostability compared to the parent cutinase.

Hydrolysis of BETEB

The thermostability of the *H. insolens* cutinase and three of the above variants was measured by hydrolysis of BETEB at 60°C for 2 hours. The hydrolysis was carried out at the above conditions, except that the temperature was fixed at 60°C and the cutinase dosage was varied. The results below are shown in the table below.

LU/ml	Variant	Variant	Variant	Parent
0	0 %	0 %	0 %	0 %
10	97 %	99 %	9 %	6 %
20	98 %	99 %	74 %	
50	98 %	94 %	93 %	15 %
100	88 %	69 %	92 %	34 %
300				41 %
600				63 %
1200				82 %

The results show a much faster hydrolysis at 60°C with the variants than with the parent cutinase.

Example 4: Hydrolysis of c3ET

The *H. insolens* cutinase and five of the above variants were tested in hydrolysis of c3ET at elevated temperature. For each cutinase, the following mixture was incubated for 2 hours at various temperatures.

0.115mg c3ET (0.1ml of 2mM c3ET dissolved in HFIP was taken in reaction vessel. Solvent was removed under vacuum, then dried up at 70°C over night)

0.1ml 0.5M glycyl-glycine buffer (pH8.5)

0.1ml enzyme solution (approx. 600LU/ml)

0.8ml Milli Q water

After the incubation, 2ml of 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) was added to each reaction mixture, then hydrolysis ratio was measured by HPLC. The results shown in Fig 3 clearly indicate that the variants have improved thermostability compared to the parent cutinase.

Example 5: Hydrolysis of c3ET on yarn

The thermostability of the *H. insolens* cutinase five of the above variants was tested using polyester yarn containing c3ET as by product. The following substrate mixture was preincubated at 60 or 65°C:

0.1g polyester yarn

0.2ml 0.5M glycyl-glycine buffer (pH8.5)

1.7ml Milli Q water

After preincubation, 0.1ml enzyme solution (approx. 1000 LU/ml) was added to each reaction vessel and incubated for 17 hours. Then 2ml HFIP was added and left for 30 minutes to extract and hydrolyze c3ET sitting on the surface of the polyester yarn; then the hydrolysis ratio was measured. The results are shown in Fig. 4.

It is seen that the variants are more effective than the parent cutinase for hydrolyzing c3ET on polyester yarn. One variant gives higher hydrolysis ratio at 65°C than at 60°C.

Example 6: Treatment of yarn with cutinase variant

Time courses of c3ET hydrolysis on polyester yarn at different temperature or dosage were examined. Time course at different temperatures is shown in Fig 5. It is seen that the optimum temperature is 65°C. At 70°C there is still about half of the activity left. Time course with increased enzyme dosage is shown in Fig 6. The curves at dosage 275 and 550 LU/ml are seen to be the same, indicating that the hydrolysis ratio reached to plateau between dosage of 100 to 275 LU/ml. Presumably 200LU/ml is enough.

Example 7: Dyeing polyester with reactive dye

The following polyester fabrics were treated:

woven fabric; ca. 2 x 2 cm, 34mg

knitted fabric; ca. 1.5 x 1.5 cm, 50mg

Each fabric was soaked in 0.9 ml, 50 mM GlyGly (glycyl-glycine) buffer (pH 8.5) and 0.1 ml solution of a variant of the *H. insolens* cutinase (1100 LU/ml), and incubated at 65 or 70°C. After one day, another 0.1 ml enzyme solution was added, incubation was continued for two more days, the fabrics were then taken out and rinsed in water. A comparative experiment was made with the parent cutinase, and a blank was treated in the same manner without enzyme.

The fabrics were stirred in a mixture of 9 g 120 g Na_2SO_4 and 60 g Na_2CO_3 in 3 liter deionized water at 60 °C for 30 min, and then rinsed with running warm water. The reactive dye was Celmazol Brilliant Blue B (product of Mitsui Chemical Co., Japan), which has the structure Chromophore-NHPh-$SO_2CH_2CH_2OSO_3Na$.

In all four experiments, (woven and knitted, 65 and 70°C), the fabrics were uniformly dyed.

Example 8: Solubilization of polyester fragments from knitted textile

A 1x1 cm sample of knitted polyester textile (PET, polymer of ethyleneglycol and terephthalic acid) was incubated for 1 hour in 1 ml of buffer at pH 10, 60°C with 0.01 mg of a variant of *H. insolens* cutinase. The reaction mixture was separated, and the release of terephthalic acid was found by measuring OD at 250 nm (ex-

pressed as OD_{250}/mg PET). comparative experiments were made without enzyme or with the parent cutinase. Results:

	Enzyme	OD_{250}
Invention	Cutinase variant	4.5
Reference	Parent cutinase	0.3
	None	0.1

The results show that the variant is effective in solubilizing polyester.

In another experiment, the cutinase variant was tested for 2 hours at 65°C with and without the addition of a non-ionic surfactant (alcohol ethoxylate, product name Softanol 50), using various amounts of the variant from 0.5 to 200 LU/ml. The results showed more solubilization in the presence of non-ionic surfactant.

Example 9: Hydrolysis of polycaprolactone and polyester film

About 0.1 g of polycaprolactone or polyester film were put in tubes. They were soaked in 5ml of 50mM GlyGly buffer (pH 8.5) with or without a variant of *H. insolens* cutinase (450 LU). They were incubated at 70°C for 5 hours. After the reaction we observed a thin layer of hydrolysate on the surface of the tubes with enzyme, both with polycaprolactone and with polyester film. On the other hand no change was observed in controls without enzyme. In the case of polycaprolactone there was 10% of weight loss. We see no weight change of polyester.

Example 10: cPET hydrolysis

The performance of a cutinase variant was compared with the parent enzyme (*H. insolens* cutinase). The trials were done as follows:

An oligomer-stained swatch of (black) PET-fabric (app. 4cm x 13cm) is subjected to the enzyme-treatment at relatively low agitation in a so-called mini-tergitometer apparatus. The PET-fabric is mounted onto a cylindrical, perforated holder (radius ca.2 cm, height ca 6 cm) , that rotates around its axis, and with the oligomer stained side of the PET fabric facing the exterior of the cylinder.

The fabric is immersed in a 150ml glass-beaker containing 100ml of the treatment solution at a given temperature (here 65°C). After a given treatment time

(here 90minutes) the PET swatch is removed from the bath and rinsed in deionized water and air dried.

After conditioning the swatches are visually ranked (with respect to oligomer stain removal) on the side having the oligomer-staining. The rating being as follows:

-2: Sample significantly worse than blank (no enzyme)

-1: Sample slightly worse than blank (no enzyme)

0: Sample can not be distinguished from blank

1: Sample slightly improved vs blank

2: Sample significantly improved over blank

Also, the swatches are read spectrofotometrically (apparatus: Hunterlab Reflectometer) to quantify the color strength (K/S-value at 600nm).

The table below summarizes the test-conditions for a trial comparing the performance the enzymes under similar conditions:

Temperature:	65°C
Buffer/pH:	50 mM glycine buffer, pH 10.3
Treatment time (min)	90
Dosage of Enzyme (LU/g)	30000

Results from the trial are summarized below

Enzyme	Visual rating (avg.)	K/S Difference @ 600 nm
None	0 (defined)	2.33
Parent cutinase	0	2.38
Cutinase variant	1.5-2.0	2.89

From this set of experiments it thus appears that the parent enzyme provides no or only very limited effect at the given test conditions (probably because the temperature is too high for the enzyme to retain activity), while the cutinase variant provides a substantial removal of the oligomer staining from the PET-fabric.

Example 11: cPET hydrolysis

The pH and temperature profile of a variant of *H. insolens* cutinase was tested in a model disperse dyeing experiment. The trials were performed as follows:

An oligomer-stained swatch of (black) PET-fabric is subjected to the conditions of a typical disperse dyeing sequence in a Werner Mathis Labomat. In overview of the process, the swatch is added to a buffer solution, heated to 130°C, cooled down to the treatment temperature. Enzyme or buffer is added and then held at the desired temperature for 30 minutes. The solution is cooled down to room temperature and turbidity in the wash liquor is measured. The reduction in turbidity is a direct measure of the cutinase activity, corresponding to hydrolyzed cPET oligomers.

Detailed description of the experiment:

A black PET (app. 4cm x 13cm) swatch is added 140 ml 100 mM Britton-Robinson buffer containing 0.2 g/l Lutensol AT11 (BASF) and loaded in the Labomat (32 rotation per minute).

The Labomat is heated to 130°C at a gradient of 9°C/minute, and held for 10 minutes.

The beakers are cooled to run temperature (according to table below) at a gradient of 9°C/minute, and held for 1 minute.

10 mL enzyme solution (100 LU/ml of the variant) or buffer solution (0 LU/ml) at appropriate pH is injected to the beakers.

The Labomat is re-heated to temperature at a gradient of 2°C/minute, and held for 30 minutes.

The swatches are removed, and the wash liquor is cooled down to room temperature.

Turbidity of the wash liquors are measured.

Evaluation: Turbidity is measured on Hach 18900 Ratio Turbidimeter (standardized with 1.8, 18, and 180 NTU Turbidity Standards). Enzyme performance is calculated relative to a blank as the difference between turbidity of blank liquor (no enzyme) and turbidity of enzyme treated liquor.

The relative performance (reduction in turbidity) of the cutinase variant is calculated, and the results are shown in the following table. When a negative num-

ber is obtained, then the result is given as "negative". A negative number is assumed to be an artifact, caused by the variation of the set up.

Temperature	pH 7	pH 8	pH 9	pH 10
60°C	39	57	37	14
65°C	39	16	60	30
70°C	25	12	54	33
75°C	22	50	114	58
85°C	negative	negative	15	negative

The results show that the cutinase variant is active over a broad pH and temperature range, with optimum oligomer removal in the current set up around pH 9 and 75°C. Inactivation seems to occur at or above 85°C.

Example 12: cPET hydrolysis

The effect of treatment time was investigated for a variant of *H. insolens* cutinase in a model disperse dyeing experiment. The trials were performed as follows:

An oligomer-stained swatch of (black) PET-fabric is subjected to the conditions of a typical disperse dyeing sequence in a Werner Mathis Labomat. In overview of the process, the swatch is added to a buffer solution, heated to 130°C, cooled down to the treatment temperature. Enzyme or buffer (100 mM Britton-Robinson pH 9) is added, and then held at 75°C for 0-40 minutes. The solution is cooled down to room temperature and turbidity in the wash liquor is measured. The reduction in turbidity is a direct measure of the cutinase activity, corresponding to hydrolyzed cPET oligomers.

Detailed description of the experiment:

A black PET (app. 4cm x 13cm) swatch is added to 140 ml 100 mM Britton-Robinson buffer containing 0.2 g/l Lutensol AT11 (BASF) and loaded in the Labomat (32 rotation per minute).

The Labomat is heated to 130°C at a gradient of 9°C/minute, and the temperature is held for 10 minutes.

The beakers are cooled to 75°C at a gradient of 9°C/minute, and held for 1 minute.

10 mL enzyme solution (100 LU/ml of variant) or 100 mM Britton-Robinson buffer pH 9.0 (0 LU/ml) is injected into the beakers.

The Labomat is re-heated to 75°C at a gradient of 2°C/minute, and held for the appropriate number of minutes (0-40 minutes, see table below).

The swatches are removed, and the wash liquor is cooled down to room temperature.

Turbidity of the wash liquors are measured.

Evaluation: Turbidity is measured on Hach 18900 Ratio Turbidimeter (standardized with 1.8, 18, and 180 NTU Turbidity Standards). Enzyme performance is calculated relative to a blank at time equal to zero: Turbidity of blank liquor at time zero (no enzyme) subtracted turbidity of enzyme treated liquor (at a given time).

The relative performance (reduction in turbidity) of the cutinase variant was calculated, and the results are shown in the following table.

Time (minutes)	Relative performance (Reduction in turbidity)
0	0
5	42
10	48
15	62
20	69
25	85
30	72
40	78

The results show that the effect of the enzyme is increased over time. At the current enzyme dose and oligomer concentration, it seems to level off above approx. 20 minutes.

Example 13: Fiber modification

The effect on wetting characteristics of a disperse dyed polyester fabric was investigated by treating the fabric with a variant of *H. insolens* cutinase prior to dye-

ing. The experiment therefore consisted of two phases, the actual fiber modification and the disperse dyeing pro- cedure.

Phase 1 - Fiber Modification:

Equipment:	Atlas Launder-O-meter LP2
Fabric:	knit 100 % scoured polyester from Testfabrics
pH:	50 mM potassium phosphate buffer, pH 7
Abrasives:	5 big steel balls
Beaker Vol.:	120 mL
Treatment:	2 hours 65°C then ramped up to 90°C and held for 1 hour
Swatch Prep:	Cut 3* 1.5 g swatch of fabric, 3 per beaker = 4.5 g

Rinse:

Rinse in deionized water.

Phase 2 - Dyeing - disperse dye:

Dye Solution:

Add together with deionized water to make liquor ratio 1:20-

0.4 % Dianix Red (DyStar) SE-CB (owf)

pH to 4.5 - 5

Dyeing Procedure:

1. One swatch per treatment from the fiber modification is used for the dyeing (1.5 g/swatch is used for the liquor ratio calculation).

2. Make dyebath according to the recipe above. Add the cold dye solution to the Labomat beakers and heat to 55°C at a gradient of 3.5°C/minute. Run for 5 minutes once temperature has been reached.

3. Add the fabric to the beaker.

4. Raise temperature to 130°C at a gradient of 1.5°C/minute. Dye for 30 minutes.

5. Cool to 70°C at a gradient of 5°C/minute. Drop bath, but collect, and rinse fabric hot (60°C) for 10 minutes. Follow the hot rinse with a room temperature overflow rinse until all bleeding had stopped.

6. Let air dry overnight.

Tests/Analysis:

AATCC Test Method 61 - Colorfastness to washing

Percent Dyebath Exhaustion - Spectrophotometer

K/S and L* - Reflectometer

AATCC TM-79 Drop Test

Results:

The results from the fiber modification are shown in the following table.

Variant dosage	Staining (AATCC TM-61)	Color Change (K/S @ 530 before and after TM-61)	Drop Test (AATCC TM-79)
Blank	4.5	5	53 sec.
50 LU/mL	4.5	5	18 sec.
100 LU/mL	4.5	5	15 sec.

The results show that the treatment of polyester with the variant increases the wetting substantially. No adverse effects are noticed on the dyeability with the disperse dye in the current set-up.

Example 14: Malodor reduction in textiles soiled with human sweat/sebum by use of a cutinase variant in laundry

The performance of cutinase, with respect to malodor reduction, can be tested in a one-cycle washing trial carried out in a Terg-O-tometer.

Experimental conditions:

Washing liquor: 1000 ml per beaker

Swatches: 100 % polyester (interlock knitted, previously cleaned by Soxhlet extraction). 24 swatches (3.3 × 3.5 cm) per beaker.

Soil: Human male axillary sweat and sebum applied by wiping the armpits after exercise.

Detergent: 5 g/L of a standard color detergent. No pH adjustment.

Water hardness: 3.2 mM Ca^{2+}/Mg^{2+} (in a ratio of 5:1)

Wash Temperature: 30°C

Wash time: 30 min

Rinse: 15 minutes in running tap water

Evaluation:

After wash the wet swatches are placed in closed, tinted 200 ml glasses. A trained sensory panel (9-11 judges) evaluates the odor by sniffing the headspace over the wet samples and evaluates the total odor intensity. The odor intensity is noted by placing a mark on an unstructured line scale measuring 15 cm, with word anchors at each end ('nothing' at the beginning of the scale and 'very strong' at the end). All evaluations are performed twice. The swatches are evaluated on day 1, 2 and 3 after wash (swatches are kept in the glasses at all times).

CLAIMS

1. A variant of a parent fungal cutinase, which variant:
   a) comprises substitution of one or more amino acid residues at a position which is located:
      i) within 17 Å from the location of the N-terminal amino acid (as calculated from amino acid residues in a crystal structure), and/or
      ii) within 20 positions from the N-terminal amino acid, and
   b) is more thermostable than the parent cutinase.

2. The variant of the preceding claim which comprises substitution of one or more amino acid residues at a position which is located:
      i) within 12 Å from the location of the N-terminal amino acid (as calculated from amino acid residues in a crystal structure), and/or
      ii) within 15 positions from the N-terminal amino acid.

3. A variant of a parent fungal cutinase comprising substitution of one or more amino acid residues which is located:
   a) within 17 Å from the location of the N-terminal amino acid (as calculated from amino acid residues in a crystal structure), and/or
   b) within 20 positions from the N-terminal amino acid,

   with the proviso that it is not a variant of the cutinase of *Fusarium solani pisi* having one of the substitutions R17, T18, T19V, D21N, I24E, Y38F, R40, G41A, S42, T43, E44, T45, G46, N47R, G49, T50, L51, P53, S54, A56C, S57, N58R, S61, A62E, K65A, D66S, G67D, W69Y, I70C, G74, G75, R78, Y119, G192, P193, D194R, A195, R196, G197V, or A199C (*Fusarium solani pisi* cutinase numbering).

4. A variant of a parent fungal cutinase comprising substitution of one or more amino acid residues which:
   a) has a solvent accessible surface, and

b) is located:

i) within 17 Å from the location of the N-terminal amino acid (as calculated from amino acid residues in a crystal structure), and/or

ii) within 20 positions from the N-terminal amino acid,

with the proviso that it is not a variant of the cutinase of *Fusarium solani pisi* having one of the substitutions T18, Y38F, R40, G41A, S42, T43, E44, T45, N47R, G49, T50, L51, P53, S54, A56C, A62E or G192 (*Fusarium solani pisi* cutinase numbering).

5. A variant of a parent fungal cutinase comprising substitution of one or more amino acid residues which is located:

a) less than 12 Å from the location of the N-terminal amino group (as calculated from amino acid residues in a crystal structure), and/or

b) within 15 positions from the N-terminal amino acid,

with the proviso that the variant is not the cutinase of *Fusarium solani pisi* having one of the substitutions R17, T18, T19V, D21N, Y38F, R40, T45, G46, N47R, G49, T50, L51, P53, S54, A56C, S57, N58R, K65A or I70C (*Fusarium solani pisi* cutinase numbering).

6. The variant of any preceding claim wherein the parent cutinase is native to a filamentous fungus, preferably a strain of *Humicola* or *Fusarium*, preferably *H. insolens* or *F. solani pisi*, most preferably *H. insolens* strain DSM 1800.

7. The variant of any preceding claim wherein the parent cutinase has an amino acid sequence which can be aligned with the cutinase of *H. insolens* strain DSM 1800.

8. The variant of any preceding claim wherein the parent cutinase has an amino acid sequence which is at least 50 % homologous to the cutinase of *H. insolens* strain DSM 1800, preferably at least 70 % homologous, more preferably at least 80 % homologous.

9. A variant of a parent fungal cutinase from *Humicola insolens* which comprises substitution of one or more amino acid residues located:

a) within 17 Å from the location of the N-terminal amino acid (as calculated from amino acid residues in a crystal structure), and/or

b) within 20 positions from the N-terminal amino acid.

10. The variant of the preceding claim which comprises substitution of one or more amino acid residues located:

a) less than 12 Å from the location of the N-terminal amino group (as calculated from amino acid residues in a crystal structure), and/or

b) within 15 positions from the N-terminal amino acid

11. The variant of any preceding claim which comprises substitution of one or more amino acids having a solvent accessible surface.

12. The variant of any preceding claim wherein one or more substitutions is substitution of a negatively charged amino acid with a neutral or positively charged amino acid or substitution of a neutral amino acid with a positively charged amino acid.

13. The variant of the preceding claim wherein one or more substitutions is at a position corresponding to position E6, E10, E30, E47, D63, E82 and/or E179 in the cutinase of *Humicola insolens* strain DSM 1800, preferably a substitution with R/K/Y/H/Q/N, more preferably a substitution corresponding to E6N/Q, E10N/Q, E47K/R and/or E179N/Q (*H. insolens* cutinase numbering).

14. The variant of any preceding claim wherein one or more substitutions is substitution with a Pro residue, preferably at a position corresponding to position A14 and/or R51.

15. The variant of any preceding claim which has one, two, three, four, five or six of said substitutions.

16. The variant of any preceding claim which has substitutions corresponding to one of the following in the cutinase of *Humicola insolens* strain DSM 1800:

a) R51P
b) E6N/Q + L138I
c) A14P + E47K
d) E47K
e) E179N/Q
f) E6N/Q + E47K + R51P
g) A14P + E47K + E179N/Q
h) E47K + E179N/Q
i) E47K + D63N
j) E6N/Q + A14P + E47K + R51P + E179N/Q
k) E6N/Q + E10N/Q + A14P + E47K + R51P + E179N/Q, or
l) Q1P + L2V + S11C + N15T + F24Y + L46I + E47K

17. The variant of any preceding claim which has hydrolytic activity towards terephthalic acid esters, particularly towards cyclic tri(ethylene terephthalate) and/or Terephthalic acid bis(2-hydroxyethyl)ester dibenzoate (BETEB).

18. The variant of any preceding claim which has a denaturation temperature which is at least 5° higher than the parent cutinase, preferably measured at pH 8.5

19. A DNA sequence encoding the variant of any preceding claim.

20. A vector comprising the DNA sequence of the preceding claim.

21. A transformed host cell harboring the DNA sequence of claim 19 or the vector of claim 20.

22. A method of producing the variant of any of claims 1-18 comprising

a) cultivating the cell of claim 21 so as to express and preferably secrete the variant, and

b) recovering the variant.

23. A method of constructing a cutinase variant, which method comprises:

a) selecting a parent fungal cutinase,

b) identifying one or more amino acid residues in the parent cutinase at positions which are:

i) within 17 Å from the location of the N-terminal amino acid (as calculated from amino acid residues in a crystal structure), and/or

ii) within 20 positions from the N-terminal amino acid, and

c) making alterations each of which is an insertion, a deletion or a substitution of the amino acid residue,

d) optionally, making alterations each of which is an insertion, a deletion or a substitution of an amino acid residue at one or more positions other than b),

e) preparing the variant resulting from steps b-d,

f) testing the thermostability of the variant,

g) optionally repeating steps b-f, and

h) selecting a variant having higher thermostability than the parent cutinase.

24. A method of producing a cutinase variant, which method comprises:

a) selecting a parent fungal cutinase,

b) identifying one or more amino acid residues in the parent cutinase at positions which are:

i) within 17 Å from the location of the N-terminal amino acid (as calculated from amino acid residues in a crystal structure), and/or

ii) within 20 positions from the N-terminal amino acid, and

c) making alterations each of which is an insertion, a deletion or a substitution of the amino acid residue,

d) optionally, making alterations each of which is an insertion, a deletion or a substitution of an amino acid residue at one or more positions other than b),

e) preparing the variant resulting from steps b-d,

f) testing the thermostability of the variant,

g) optionally repeating steps b-f,

h) selecting a variant having higher thermostability than the parent cutinase, and

i) producing the variant to obtain the cutinase variant.

25. A process for enzymatic hydrolysis of a cyclic oligomer of poly(ethylene terephthalate), which process comprises treating the cyclic oligomer with a variant of a parent fungal cutinase, which variant comprises substitution of one or more amino acid residues at a position which is located:

i) within 17 Å from the location of the N-terminal amino acid (as calculated from amino acid residues in a crystal structure), and/or

ii) within 20 positions from the N-terminal amino acid.

26. The process of the preceding claim, in which the cyclic oligomer is cyclic tri(ethylene terephthalate).

27. The process of claim 25 or 26 wherein the treatment is done at 60-80°C, preferably at 65-75°C.

28. The process of any of claims 25-27 wherein the cyclic oligomer is present in and on the fibers of a polyester containing fabric or yarn.

29. The process of any of claims 25-28 which further comprises subsequently rinsing the fabric or yarn, preferably rinsing with an aqueous solution having a pH in the range of from about pH 7 to about pH 11.

30. A process for dyeing polyester fabric or yarn, comprising:

a) treating the fabric or yarn with a cutinase having a thermal denaturation temperature of 65°C or higher at pH 8.5; and

b) dyeing the treated fabric with a reactive dye or a disperse dye.

31. The process of the preceding claim wherein the cutinase is the variant of any of claims 1-18.

32. A detergent composition comprising a surfactant and the variant of any of claims 1-18.

33. A method for detecting cutinase activity in a sample, comprising incubating the sample with terephthalic acid bis(2-hydroxyethyl)ester dibenzoate and detecting hydrolysis of said ester.

34. A process for improving the functional finish of a PET-containing yarn or fabric comprising

a) treating the yarn or fabric with the variant of any of claims 1-18, and

b) subsequently the yarn or fabric with a finishing agent selected from the group consisting of softeners, anti-crease resins, anti-static agents, anti-soiling agents.

Fig. 1

3D structure of cutinase from *Humicola insolens*

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ATOM      1  N   GLY A   3      24.424  -7.935  18.390  1.00 46.73
ATOM      2  CA  GLY A   3      23.848  -8.994  17.546  1.00 42.29
ATOM      3  C   GLY A   3      24.396 -10.112  16.727  1.00 37.35
ATOM      4  O   GLY A   3      25.347 -10.913  16.728  1.00 35.38
ATOM      5  N   ALA A   4      23.664 -10.625  15.797  1.00 34.53
ATOM      6  CA  ALA A   4      23.051 -10.874  14.555  1.00 30.95
ATOM      7  C   ALA A   4      21.574 -11.246  14.920  1.00 28.33
ATOM      8  O   ALA A   4      20.677 -10.499  14.446  1.00 22.94
ATOM      9  CB  ALA A   4      23.574 -11.780  13.556  1.00 26.92
ATOM     10  N   ILE A   5      21.583 -12.058  16.043  1.00 26.48
ATOM     11  CA  ILE A   5      20.281 -12.289  16.637  1.00 25.65
ATOM     12  C   ILE A   5      20.316 -12.151  18.118  1.00 22.40
ATOM     13  O   ILE A   5      21.060 -12.888  18.717  1.00 24.74
ATOM     14  CB  ILE A   5      19.724 -13.683  16.524  1.00 26.04
ATOM     15  CG1 ILE A   5      19.852 -13.927  15.050  1.00 29.85
ATOM     16  CG2 ILE A   5      18.374 -13.558  17.159  1.00 20.48
ATOM     17  CD1 ILE A   5      19.066 -15.133  14.709  1.00 27.96
ATOM     18  N   GLU A   6      19.461 -11.377  18.668  1.00 20.52
ATOM     19  CA  GLU A   6      19.207 -11.015  20.040  1.00 17.94
ATOM     20  C   GLU A   6      17.711 -11.027  20.432  1.00 17.76
ATOM     21  O   GLU A   6      16.931 -10.165  19.990  1.00 17.60
ATOM     22  CB  GLU A   6      19.809  -9.614  20.199  1.00 14.22
ATOM     23  CG  GLU A   6      21.232  -9.374  20.385  1.00 16.71
ATOM     24  CD  GLU A   6      22.148 -10.387  21.030  1.00 34.47
ATOM     25  OE1 GLU A   6      21.634 -11.347  21.693  1.00 49.57
ATOM     26  OE2 GLU A   6      23.410 -10.310  20.975  1.00 37.43
ATOM     27  N   ASN A   7      17.375 -11.895  21.333  1.00 21.67
ATOM     28  CA  ASN A   7      16.070 -11.854  21.846  1.00 24.04
ATOM     29  C   ASN A   7      15.927 -11.488  23.238  1.00 22.08
ATOM     30  O   ASN A   7      15.098 -12.179  23.820  1.00 24.00
ATOM     31  CB  ASN A   7      15.468 -13.307  21.820  1.00 25.06
ATOM     32  CG  ASN A   7      15.039 -13.160  20.341  1.00 38.52
ATOM     33  OD1 ASN A   7      15.519 -14.147  19.759  1.00 48.45
ATOM     34  ND2 ASN A   7      14.318 -12.081  19.968  1.00 36.89
ATOM     35  N   GLY A   8      16.671 -10.813  23.926  1.00 23.56
ATOM     36  CA  GLY A   8      16.654 -10.628  25.363  1.00 23.69
ATOM     37  C   GLY A   8      15.366 -10.247  25.984  1.00 22.72
ATOM     38  O   GLY A   8      14.967 -10.939  26.867  1.00 32.25
ATOM     39  N   LEU A   9      14.785  -9.144  25.755  1.00 23.61
ATOM     40  CA  LEU A   9      13.470  -8.753  26.033  1.00 23.73
ATOM     41  C   LEU A   9      12.559  -9.961  25.782  1.00 25.93
ATOM     42  O   LEU A   9      11.494 -10.054  26.480  1.00 30.47
ATOM     43  CB  LEU A   9      12.971  -7.621  25.105  1.00  5.84
ATOM     44  CG  LEU A   9      11.556  -7.227  25.470  1.00 23.25
ATOM     45  CD1 LEU A   9      11.422  -6.765  26.968  1.00 20.21
ATOM     46  CD2 LEU A   9      11.009  -6.071  24.714  1.00 17.64
ATOM     47  N   GLU A  10      12.775 -10.786  24.773  1.00 29.56
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ATOM     48  CA  GLU A  10      11.635 -11.681  24.484  1.00 33.93
ATOM     49  C   GLU A  10      11.640 -12.872  25.412  1.00 32.18
ATOM     50  O   GLU A  10      10.600 -13.159  25.996  1.00 36.67
ATOM     51  CB  GLU A  10      11.513 -11.996  23.012  1.00 40.97
ATOM     52  CG  GLU A  10      10.054 -12.303  22.745  1.00 51.96
ATOM     53  CD  GLU A  10       9.570 -11.711  21.437  1.00 54.08
ATOM     54  OE1 GLU A  10      10.488 -11.440  20.635  1.00 48.22
ATOM     55  OE2 GLU A  10       8.323 -11.643  21.471  1.00 52.39
ATOM     56  N   SER A  11      12.822 -13.334  25.688  1.00 29.58
ATOM     57  CA  SER A  11      12.993 -14.455  26.645  1.00 35.25
ATOM     58  C   SER A  11      13.403 -14.012  28.047  1.00 39.86
ATOM     59  O   SER A  11      13.688 -14.790  28.919  1.00 43.72
ATOM     60  CB  SER A  11      14.053 -15.364  25.983  1.00 33.73
ATOM     61  OG  SER A  11      15.275 -14.620  25.928  1.00 46.98
ATOM     62  N   GLY A  12      13.467 -12.802  28.456  1.00 41.40
ATOM     63  CA  GLY A  12      13.841 -12.332  29.752  1.00 45.34
ATOM     64  C   GLY A  12      12.673 -12.562  30.694  1.00 47.62
ATOM     65  O   GLY A  12      11.485 -12.335  30.335  1.00 50.76
ATOM     66  N   SER A  13      12.969 -12.900  31.936  1.00 48.09
ATOM     67  CA  SER A  13      11.974 -13.158  32.995  1.00 45.26
ATOM     68  C   SER A  13      11.509 -11.933  33.772  1.00 39.53
ATOM     69  O   SER A  13      12.563 -11.204  33.992  1.00 36.30
ATOM     70  CB  SER A  13      12.708 -14.006  34.101  1.00 51.20
ATOM     71  OG  SER A  13      12.006 -13.947  35.338  1.00 57.14
ATOM     72  N   ALA A  14      10.256 -11.785  34.214  1.00 35.22
ATOM     73  CA  ALA A  14      10.068 -10.530  34.964  1.00 34.78
ATOM     74  C   ALA A  14      10.574 -10.620  36.417  1.00 37.51
ATOM     75  O   ALA A  14      10.809  -9.584  37.113  1.00 38.41
ATOM     76  CB  ALA A  14       8.714  -9.915  34.903  1.00 32.71
ATOM     77  N   ASN A  15      11.039 -11.834  36.737  1.00 38.85
ATOM     78  CA  ASN A  15      11.715 -12.086  37.963  1.00 43.49
ATOM     79  C   ASN A  15      13.073 -11.411  37.953  1.00 46.45
ATOM     80  O   ASN A  15      13.453 -11.022  39.022  1.00 52.50
ATOM     81  CB  ASN A  15      12.088 -13.533  38.207  1.00 53.08
ATOM     82  CG  ASN A  15      10.772 -14.226  38.553  1.00 71.86
ATOM     83  OD1 ASN A  15       9.837 -13.535  38.998  1.00 71.73
ATOM     84  ND2 ASN A  15      10.866 -15.523  38.267  1.00 77.71
ATOM     85  N   ALA A  16      13.712 -11.305  36.812  1.00 46.73
ATOM     86  CA  ALA A  16      14.915 -10.470  36.743  1.00 41.22
ATOM     87  C   ALA A  16      15.031  -9.286  35.798  1.00 36.70
ATOM     88  O   ALA A  16      16.027  -9.254  35.075  1.00 37.67
ATOM     89  CB  ALA A  16      15.903 -11.545  36.301  1.00 41.80
ATOM     90  N   CYS A  17      14.300  -8.227  35.843  1.00 30.62
ATOM     91  CA  CYS A  17      14.614  -7.093  34.997  1.00 31.78
ATOM     92  C   CYS A  17      16.024  -6.579  35.149  1.00 32.94
ATOM     93  O   CYS A  17      16.744  -6.850  36.113  1.00 39.10
ATOM     94  CB  CYS A  17      13.679  -5.881  35.138  1.00 28.00
ATOM     95  SG  CYS A  17      12.048  -6.583  34.858  1.00 24.72
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ATOM	96	N	PRO	A	18	16.529	-5.910	34.092	1.00	30.49
ATOM	97	CA	PRO	A	18	17.994	-5.626	33.971	1.00	22.04
ATOM	98	C	PRO	A	18	18.178	-4.138	34.241	1.00	20.15
ATOM	99	O	PRO	A	18	17.085	-3.459	34.370	1.00	17.83
ATOM	100	CB	PRO	A	18	18.353	-6.003	32.559	1.00	19.20
ATOM	101	CG	PRO	A	18	17.044	-6.595	32.101	1.00	20.16
ATOM	102	CD	PRO	A	18	15.903	-5.936	32.792	1.00	24.35
ATOM	103	N	ASP	A	19	19.428	-3.652	34.011	1.00	14.85
ATOM	104	CA	ASP	A	19	19.451	-2.168	34.226	1.00	16.59
ATOM	105	C	ASP	A	19	18.739	-1.367	33.156	1.00	20.42
ATOM	106	O	ASP	A	19	18.311	-0.242	33.430	1.00	23.84
ATOM	107	CB	ASP	A	19	20.896	-1.818	34.485	1.00	27.25
ATOM	108	CG	ASP	A	19	21.433	-2.389	35.793	1.00	42.30
ATOM	109	OD1	ASP	A	19	21.162	-3.549	36.297	1.00	53.52
ATOM	110	OD2	ASP	A	19	22.251	-1.719	36.543	1.00	54.02
ATOM	111	N	ALA	A	20	18.646	-1.780	31.895	1.00	20.18
ATOM	112	CA	ALA	A	20	18.066	-1.036	30.809	1.00	17.43
ATOM	113	C	ALA	A	20	17.713	-2.087	29.703	1.00	16.06
ATOM	114	O	ALA	A	20	18.334	-3.172	29.860	1.00	9.45
ATOM	115	CB	ALA	A	20	18.975	-0.048	30.100	1.00	12.07
ATOM	116	N	ILE	A	21	16.814	-1.602	28.829	1.00	8.47
ATOM	117	CA	ILE	A	21	16.657	-2.583	27.753	1.00	9.23
ATOM	118	C	ILE	A	21	16.952	-1.745	26.486	1.00	14.77
ATOM	119	O	ILE	A	21	16.681	-0.473	26.403	1.00	12.01
ATOM	120	CB	ILE	A	21	15.208	-2.984	27.837	1.00	16.28
ATOM	121	CG1	ILE	A	21	14.851	-3.898	28.956	1.00	15.55
ATOM	122	CG2	ILE	A	21	14.689	-3.671	26.514	1.00	13.71
ATOM	123	CD1	ILE	A	21	13.401	-3.879	29.372	1.00	6.12
ATOM	124	N	LEU	A	22	17.432	-2.451	25.391	1.00	12.24
ATOM	125	CA	LEU	A	22	17.665	-1.774	24.087	1.00	11.27
ATOM	126	C	LEU	A	22	16.849	-2.517	23.038	1.00	14.60
ATOM	127	O	LEU	A	22	16.908	-3.781	22.850	1.00	9.78
ATOM	128	CB	LEU	A	22	19.087	-1.865	23.693	1.00	10.96
ATOM	129	CG	LEU	A	22	19.493	-1.543	22.257	1.00	10.32
ATOM	130	CD1	LEU	A	22	19.311	-0.081	21.900	1.00	4.72
ATOM	131	CD2	LEU	A	22	20.990	-1.842	22.156	1.00	7.42
ATOM	132	N	ILE	A	23	16.038	-1.815	22.242	1.00	15.13
ATOM	133	CA	ILE	A	23	15.298	-2.459	21.115	1.00	18.06
ATOM	134	C	ILE	A	23	15.916	-1.771	19.901	1.00	17.42
ATOM	135	O	ILE	A	23	16.117	-0.519	19.795	1.00	19.31
ATOM	136	CB	ILE	A	23	13.820	-2.194	21.392	1.00	18.16
ATOM	137	CG1	ILE	A	23	13.208	-3.076	22.447	1.00	14.23
ATOM	138	CG2	ILE	A	23	12.787	-2.167	20.247	1.00	13.19
ATOM	139	CD1	ILE	A	23	12.142	-2.065	22.976	1.00	20.41
ATOM	140	N	PHE	A	24	16.218	-2.548	18.940	1.00	14.59
ATOM	141	CA	PHE	A	24	16.859	-2.159	17.671	1.00	11.72
ATOM	142	C	PHE	A	24	16.347	-2.719	16.353	1.00	7.25
ATOM	143	O	PHE	A	24	16.095	-3.998	16.161	1.00	3.47

ATOM	144	CB	PHE	A	24	18.195	-2.855	17.658	1.00	12.61
ATOM	145	CG	PHE	A	24	19.015	-2.150	16.716	1.00	10.72
ATOM	146	CD1	PHE	A	24	19.457	-0.844	16.913	1.00	13.08
ATOM	147	CD2	PHE	A	24	19.325	-2.852	15.558	1.00	6.61
ATOM	148	CE1	PHE	A	24	20.232	-0.187	15.983	1.00	4.86
ATOM	149	CE2	PHE	A	24	20.061	-2.218	14.545	1.00	7.61
ATOM	150	CZ	PHE	A	24	20.550	-0.823	14.804	1.00	8.78
ATOM	151	N	ALA	A	25	16.037	-1.700	15.449	1.00	6.32
ATOM	152	CA	ALA	A	25	15.662	-2.158	14.068	1.00	7.18
ATOM	153	C	ALA	A	25	16.851	-1.976	13.055	1.00	8.59
ATOM	154	O	ALA	A	25	17.518	-1.000	13.133	1.00	5.95
ATOM	155	CB	ALA	A	25	14.488	-1.402	13.562	1.00	8.27
ATOM	156	N	ARG	A	26	17.174	-3.032	12.325	1.00	8.84
ATOM	157	CA	ARG	A	26	18.134	-3.278	11.277	1.00	4.04
ATOM	158	C	ARG	A	26	17.691	-2.694	9.894	1.00	7.67
ATOM	159	O	ARG	A	26	16.527	-2.361	9.525	1.00	9.36
ATOM	160	CB	ARG	A	26	18.581	-4.659	10.756	1.00	6.06
ATOM	161	CG	ARG	A	26	17.705	-5.741	10.439	1.00	5.08
ATOM	162	CD	ARG	A	26	18.069	-7.224	10.382	1.00	6.73
ATOM	163	NE	ARG	A	26	17.000	-8.053	9.708	1.00	9.04
ATOM	164	CZ	ARG	A	26	15.724	-8.206	9.912	1.00	7.06
ATOM	165	NH1	ARG	A	26	15.085	-7.535	10.895	1.00	22.93
ATOM	166	NH2	ARG	A	26	14.809	-8.825	9.346	1.00	7.89
ATOM	167	N	GLY	A	27	18.761	-2.539	9.092	1.00	7.71
ATOM	168	CA	GLY	A	27	18.537	-1.888	7.782	1.00	5.34
ATOM	169	C	GLY	A	27	18.063	-2.896	6.862	1.00	4.70
ATOM	170	O	GLY	A	27	18.155	-4.139	7.075	1.00	13.14
ATOM	171	N	SER	A	28	17.562	-2.612	5.765	1.00	11.82
ATOM	172	CA	SER	A	28	17.108	-3.325	4.615	1.00	14.72
ATOM	173	C	SER	A	28	18.214	-4.327	4.142	1.00	7.74
ATOM	174	O	SER	A	28	19.286	-3.973	4.083	1.00	6.71
ATOM	175	CB	SER	A	28	16.460	-2.352	3.538	1.00	6.38
ATOM	176	OG	SER	A	28	16.819	-0.978	3.833	1.00	28.10
ATOM	177	N	THR	A	29	17.942	-5.634	4.241	1.00	4.79
ATOM	178	CA	THR	A	29	18.562	-6.763	3.914	1.00	8.71
ATOM	179	C	THR	A	29	19.500	-7.271	4.985	1.00	14.00
ATOM	180	O	THR	A	29	20.162	-8.326	4.713	1.00	17.68
ATOM	181	CB	THR	A	29	19.454	-6.680	2.617	1.00	14.90
ATOM	182	OG1	THR	A	29	20.736	-6.066	2.595	1.00	14.00
ATOM	183	CG2	THR	A	29	18.785	-5.888	1.561	1.00	15.59
ATOM	184	N	GLU	A	30	19.740	-6.599	6.105	1.00	14.52
ATOM	185	CA	GLU	A	30	20.677	-7.266	7.056	1.00	14.10
ATOM	186	C	GLU	A	30	20.092	-8.513	7.647	1.00	13.07
ATOM	187	O	GLU	A	30	18.916	-8.726	7.705	1.00	19.98
ATOM	188	CB	GLU	A	30	21.228	-6.371	8.072	1.00	15.45
ATOM	189	CG	GLU	A	30	21.166	-4.945	7.709	1.00	8.37
ATOM	190	CD	GLU	A	30	22.073	-4.143	8.637	1.00	23.08
ATOM	191	OE1	GLU	A	30	21.395	-3.328	9.284	1.00	19.26

ATOM	192	OE2	GLU	A	30	23.317	-4.327	8.712	1.00	19.71
ATOM	193	N	PRO	A	31	20.875	-9.479	7.918	1.00	13.09
ATOM	194	CA	PRO	A	31	20.477	-10.818	8.402	1.00	14.56
ATOM	195	C	PRO	A	31	20.167	-10.698	9.895	1.00	18.27
ATOM	196	O	PRO	A	31	20.148	-9.636	10.392	1.00	20.45
ATOM	197	CB	PRO	A	31	21.690	-11.692	8.215	1.00	10.95
ATOM	198	CG	PRO	A	31	22.790	-10.664	8.455	1.00	11.24
ATOM	199	CD	PRO	A	31	22.350	-9.316	7.864	1.00	13.71
ATOM	200	N	GLY	A	32	19.612	-11.689	10.472	1.00	18.99
ATOM	201	CA	GLY	A	32	19.205	-11.774	11.816	1.00	13.53
ATOM	202	C	GLY	A	32	18.133	-10.808	12.188	1.00	16.62
ATOM	203	O	GLY	A	32	17.345	-10.294	11.411	1.00	17.01
ATOM	204	N	ASN	A	33	18.055	-10.528	13.468	1.00	16.15
ATOM	205	CA	ASN	A	33	17.290	-9.346	13.823	1.00	14.74
ATOM	206	C	ASN	A	33	18.294	-8.273	14.230	1.00	15.46
ATOM	207	O	ASN	A	33	17.774	-7.184	14.575	1.00	15.90
ATOM	208	CB	ASN	A	33	16.241	-9.663	14.867	1.00	17.42
ATOM	209	CG	ASN	A	33	16.827	-10.201	16.127	1.00	17.97
ATOM	210	OD1	ASN	A	33	16.112	-10.395	17.089	1.00	19.05
ATOM	211	ND2	ASN	A	33	18.074	-10.460	16.112	1.00	13.29
ATOM	212	N	MET	A	34	19.633	-8.378	14.282	1.00	14.22
ATOM	213	CA	MET	A	34	20.282	-7.171	14.751	1.00	12.97
ATOM	214	C	MET	A	34	21.142	-6.663	13.611	1.00	19.02
ATOM	215	O	MET	A	34	21.654	-5.512	13.713	1.00	26.04
ATOM	216	CB	MET	A	34	21.202	-7.329	15.859	1.00	13.39
ATOM	217	CG	MET	A	34	20.579	-7.713	17.163	1.00	9.02
ATOM	218	SD	MET	A	34	20.175	-6.316	18.069	1.00	9.13
ATOM	219	CE	MET	A	34	21.481	-5.121	18.095	1.00	4.11
ATOM	220	N	GLY	A	35	21.259	-7.446	12.550	1.00	19.99
ATOM	221	CA	GLY	A	35	22.071	-7.135	11.418	1.00	14.30
ATOM	222	C	GLY	A	35	23.511	-7.340	11.764	1.00	17.58
ATOM	223	O	GLY	A	35	23.965	-7.724	12.842	1.00	12.78
ATOM	224	N	ILE	A	36	24.450	-6.839	10.950	1.00	20.63
ATOM	225	CA	ILE	A	36	25.833	-7.029	11.277	1.00	17.71
ATOM	226	C	ILE	A	36	26.609	-5.714	11.280	1.00	16.15
ATOM	227	O	ILE	A	36	27.865	-5.618	11.662	1.00	20.30
ATOM	228	CB	ILE	A	36	26.412	-8.070	10.327	1.00	30.19
ATOM	229	CG1	ILE	A	36	26.088	-7.448	8.959	1.00	31.16
ATOM	230	CG2	ILE	A	36	25.944	-9.490	10.543	1.00	15.68
ATOM	231	CD1	ILE	A	36	26.922	-8.149	7.958	1.00	34.10
ATOM	232	N	THR	A	37	25.905	-4.589	11.040	1.00	13.00
ATOM	233	CA	THR	A	37	26.825	-3.396	11.141	1.00	9.67
ATOM	234	C	THR	A	37	26.587	-2.513	12.350	1.00	15.44
ATOM	235	O	THR	A	37	27.040	-3.055	13.410	1.00	20.20
ATOM	236	CB	THR	A	37	26.592	-2.679	9.818	1.00	14.13
ATOM	237	OG1	THR	A	37	25.241	-2.212	9.503	1.00	22.62
ATOM	238	CG2	THR	A	37	26.949	-3.739	8.800	1.00	2.29
ATOM	239	N	VAL	A	38	25.733	-1.493	12.249	1.00	11.92

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ATOM    240  CA  VAL A  38      25.237  -0.800  13.411  1.00 15.22
ATOM    241  C   VAL A  38      24.588  -1.455  14.612  1.00 14.68
ATOM    242  O   VAL A  38      24.906  -1.185  15.733  1.00 15.89
ATOM    243  CB  VAL A  38      24.124   0.180  12.855  1.00 14.13
ATOM    244  CG1 VAL A  38      23.663   0.897  14.167  1.00 13.55
ATOM    245  CG2 VAL A  38      24.570   1.025  11.670  1.00  6.75
ATOM    246  N   GLY A  39      23.745  -2.410  14.677  1.00 14.24
ATOM    247  CA  GLY A  39      23.135  -3.151  15.746  1.00 11.03
ATOM    248  C   GLY A  39      24.096  -3.586  16.791  1.00 13.34
ATOM    249  O   GLY A  39      24.131  -3.181  17.934  1.00 15.13
ATOM    250  N   PRO A  40      25.067  -4.340  16.352  1.00 14.70
ATOM    251  CA  PRO A  40      26.094  -5.025  17.171  1.00 13.44
ATOM    252  C   PRO A  40      27.010  -3.909  17.589  1.00 11.81
ATOM    253  O   PRO A  40      27.346  -3.871  18.764  1.00 12.79
ATOM    254  CB  PRO A  40      26.723  -6.111  16.279  1.00  8.43
ATOM    255  CG  PRO A  40      25.873  -6.243  14.950  1.00  4.84
ATOM    256  CD  PRO A  40      25.198  -4.902  14.995  1.00 12.36
ATOM    257  N   ALA A  41      27.226  -2.979  16.695  1.00  7.41
ATOM    258  CA  ALA A  41      28.066  -1.962  17.278  1.00 11.03
ATOM    259  C   ALA A  41      27.378  -1.206  18.439  1.00 14.87
ATOM    260  O   ALA A  41      28.028  -0.503  19.274  1.00 14.26
ATOM    261  CB  ALA A  41      28.579  -0.905  16.313  1.00  7.17
ATOM    262  N   LEU A  42      26.135  -0.811  18.237  1.00 11.87
ATOM    263  CA  LEU A  42      25.487  -0.048  19.300  1.00 12.36
ATOM    264  C   LEU A  42      25.337  -0.856  20.624  1.00 11.94
ATOM    265  O   LEU A  42      25.423  -0.397  21.730  1.00  8.33
ATOM    266  CB  LEU A  42      24.036   0.168  18.811  1.00 13.24
ATOM    267  CG  LEU A  42      23.272   1.160  19.676  1.00  6.90
ATOM    268  CD1 LEU A  42      24.108   2.419  19.962  1.00  6.62
ATOM    269  CD2 LEU A  42      21.991   1.580  18.943  1.00  7.11
ATOM    270  N   ALA A  43      24.905  -2.095  20.482  1.00 10.88
ATOM    271  CA  ALA A  43      24.761  -3.027  21.553  1.00 12.37
ATOM    272  C   ALA A  43      26.106  -3.136  22.252  1.00 15.45
ATOM    273  O   ALA A  43      25.958  -2.743  23.433  1.00 20.80
ATOM    274  CB  ALA A  43      24.148  -4.324  21.002  1.00  9.60
ATOM    275  N   ASN A  44      27.263  -3.440  21.636  1.00 16.91
ATOM    276  CA  ASN A  44      28.454  -3.434  22.439  1.00 20.33
ATOM    277  C   ASN A  44      28.717  -2.044  23.113  1.00 17.66
ATOM    278  O   ASN A  44      29.019  -1.991  24.301  1.00 17.06
ATOM    279  CB  ASN A  44      29.756  -3.695  21.625  1.00 35.48
ATOM    280  CG  ASN A  44      29.564  -5.115  21.138  1.00 58.23
ATOM    281  OD1 ASN A  44      30.013  -5.403  20.034  1.00 79.77
ATOM    282  ND2 ASN A  44      28.908  -5.945  21.921  1.00 70.10
ATOM    283  N   GLY A  45      28.682  -0.988  22.297  1.00 14.39
ATOM    284  CA  GLY A  45      29.015   0.221  22.976  1.00 11.65
ATOM    285  C   GLY A  45      28.175   0.255  24.234  1.00 14.30
ATOM    286  O   GLY A  45      28.529   0.582  25.385  1.00 10.77
ATOM    287  N   LEU A  46      26.861   0.099  24.065  1.00 16.88
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ATOM	288	CA	LEU	A	46	25.968	0.248	25.207	1.00	16.29
ATOM	289	C	LEU	A	46	26.395	-0.651	26.346	1.00	13.48
ATOM	290	O	LEU	A	46	26.579	-0.325	27.462	1.00	7.75
ATOM	291	CB	LEU	A	46	24.608	-0.243	24.847	1.00	19.46
ATOM	292	CG	LEU	A	46	23.642	0.551	25.664	1.00	13.97
ATOM	293	CD1	LEU	A	46	24.089	1.994	25.563	1.00	13.99
ATOM	294	CD2	LEU	A	46	22.275	0.465	25.038	1.00	32.18
ATOM	295	N	GLU	A	47	26.523	-1.890	25.882	1.00	15.90
ATOM	296	CA	GLU	A	47	26.910	-2.886	26.909	1.00	24.03
ATOM	297	C	GLU	A	47	28.140	-2.500	27.702	1.00	24.14
ATOM	298	O	GLU	A	47	28.722	-3.203	28.500	1.00	27.24
ATOM	299	CB	GLU	A	47	27.147	-4.206	26.204	1.00	33.33
ATOM	300	CG	GLU	A	47	27.386	-5.254	27.245	1.00	51.29
ATOM	301	CD	GLU	A	47	27.661	-6.560	26.524	1.00	68.40
ATOM	302	OE1	GLU	A	47	26.741	-7.007	25.777	1.00	66.37
ATOM	303	OE2	GLU	A	47	28.856	-6.921	26.830	1.00	78.70
ATOM	304	N	SER	A	48	28.992	-1.626	27.215	1.00	27.50
ATOM	305	CA	SER	A	48	30.331	-1.518	27.789	1.00	25.23
ATOM	306	C	SER	A	48	30.108	-0.555	28.926	1.00	26.91
ATOM	307	O	SER	A	48	31.124	-0.058	29.462	1.00	33.39
ATOM	308	CB	SER	A	48	31.116	-0.990	26.621	1.00	21.90
ATOM	309	OG	SER	A	48	31.294	0.422	26.483	1.00	27.87
ATOM	310	N	HIS	A	49	28.826	-0.101	28.995	1.00	25.04
ATOM	311	CA	HIS	A	49	28.542	0.955	29.956	1.00	19.72
ATOM	312	C	HIS	A	49	27.480	0.461	30.950	1.00	22.55
ATOM	313	O	HIS	A	49	27.186	1.089	31.898	1.00	27.93
ATOM	314	CB	HIS	A	49	28.094	2.197	29.463	1.00	16.13
ATOM	315	CG	HIS	A	49	28.806	3.036	28.520	1.00	39.79
ATOM	316	ND1	HIS	A	49	29.564	4.058	28.953	1.00	45.66
ATOM	317	CD2	HIS	A	49	28.776	3.070	27.197	1.00	46.91
ATOM	318	CE1	HIS	A	49	30.028	4.750	27.979	1.00	45.87
ATOM	319	NE2	HIS	A	49	29.544	4.139	26.934	1.00	50.84
ATOM	320	N	ILE	A	50	27.009	-0.703	30.715	1.00	18.34
ATOM	321	CA	ILE	A	50	25.874	-1.129	31.415	1.00	19.89
ATOM	322	C	ILE	A	50	25.917	-2.629	31.146	1.00	26.29
ATOM	323	O	ILE	A	50	25.322	-3.023	30.168	1.00	25.33
ATOM	324	CB	ILE	A	50	24.527	-0.535	31.008	1.00	10.50
ATOM	325	CG1	ILE	A	50	24.340	0.906	31.292	1.00	4.97
ATOM	326	CG2	ILE	A	50	23.466	-1.298	31.697	1.00	12.96
ATOM	327	CD1	ILE	A	50	23.413	1.845	30.602	1.00	16.65
ATOM	328	N	ARG	A	51	26.707	-3.256	32.066	1.00	31.77
ATOM	329	CA	ARG	A	51	26.887	-4.714	32.107	1.00	29.06
ATOM	330	C	ARG	A	51	25.457	-5.331	32.170	1.00	32.68
ATOM	331	O	ARG	A	51	25.396	-6.363	31.512	1.00	37.16
ATOM	332	N	ASN	A	52	24.380	-4.817	32.788	1.00	28.48
ATOM	333	CA	ASN	A	52	23.284	-5.767	32.832	1.00	26.39
ATOM	334	C	ASN	A	52	22.176	-5.178	31.993	1.00	27.75
ATOM	335	O	ASN	A	52	21.333	-4.488	32.636	1.00	26.68

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ATOM    336  CB  ASN A  52      22.750  -5.884  34.232  1.00 34.86
ATOM    337  CG  ASN A  52      21.637  -6.879  34.271  1.00 39.54
ATOM    338  OD1 ASN A  52      20.781  -6.541  35.095  1.00 54.31
ATOM    339  ND2 ASN A  52      21.611  -7.954  33.503  1.00 48.82
ATOM    340  N   ILE A  53      22.127  -5.699  30.800  1.00 24.42
ATOM    341  CA  ILE A  53      21.261  -5.092  29.772  1.00 20.15
ATOM    342  C   ILE A  53      20.585  -6.151  28.912  1.00 17.63
ATOM    343  O   ILE A  53      21.020  -7.349  28.917  1.00 18.01
ATOM    344  CB  ILE A  53      22.245  -4.297  28.880  1.00 14.09
ATOM    345  CG1 ILE A  53      21.682  -3.257  27.936  1.00 22.91
ATOM    346  CG2 ILE A  53      22.907  -5.321  27.946  1.00 16.37
ATOM    347  CD1 ILE A  53      22.877  -2.315  27.622  1.00 38.17
ATOM    348  N   TRP A  54      19.447  -5.880  28.383  1.00 15.19
ATOM    349  CA  TRP A  54      18.804  -6.889  27.567  1.00 17.96
ATOM    350  C   TRP A  54      18.803  -6.230  26.151  1.00 19.82
ATOM    351  O   TRP A  54      18.340  -5.059  25.985  1.00 18.37
ATOM    352  CB  TRP A  54      17.364  -7.046  27.998  1.00 23.18
ATOM    353  CG  TRP A  54      16.949  -7.932  29.100  1.00 24.57
ATOM    354  CD1 TRP A  54      17.757  -8.727  29.895  1.00 24.46
ATOM    355  CD2 TRP A  54      15.595  -8.164  29.603  1.00 30.21
ATOM    356  NE1 TRP A  54      17.004  -9.372  30.858  1.00 25.87
ATOM    357  CE2 TRP A  54      15.692  -9.039  30.700  1.00 24.92
ATOM    358  CE3 TRP A  54      14.358  -7.633  29.243  1.00 36.26
ATOM    359  CZ2 TRP A  54      14.611  -9.442  31.432  1.00 19.75
ATOM    360  CZ3 TRP A  54      13.316  -8.042  30.009  1.00 32.94
ATOM    361  CH2 TRP A  54      13.451  -8.916  31.068  1.00 23.02
ATOM    362  N   ILE A  55      19.063  -7.152  25.204  1.00 15.21
ATOM    363  CA  ILE A  55      19.178  -6.655  23.838  1.00 12.41
ATOM    364  C   ILE A  55      18.091  -7.215  22.962  1.00 11.40
ATOM    365  O   ILE A  55      17.955  -8.378  22.680  1.00  7.34
ATOM    366  CB  ILE A  55      20.546  -6.962  23.201  1.00 16.44
ATOM    367  CG1 ILE A  55      21.939  -6.409  23.702  1.00  8.75
ATOM    368  CG2 ILE A  55      20.384  -6.460  21.750  1.00 21.77
ATOM    369  CD1 ILE A  55      21.767  -5.582  24.863  1.00 16.23
ATOM    370  N   GLN A  56      17.226  -6.412  22.390  1.00  9.67
ATOM    371  CA  GLN A  56      16.161  -7.016  21.619  1.00 10.90
ATOM    372  C   GLN A  56      16.432  -6.621  20.143  1.00 13.08
ATOM    373  O   GLN A  56      16.402  -5.393  19.953  1.00 10.32
ATOM    374  CB  GLN A  56      14.786  -6.542  22.014  1.00 11.49
ATOM    375  CG  GLN A  56      13.653  -7.256  21.316  1.00 23.47
ATOM    376  CD  GLN A  56      13.789  -8.741  21.351  1.00 24.88
ATOM    377  OE1 GLN A  56      13.610  -9.379  20.324  1.00  9.56
ATOM    378  NE2 GLN A  56      14.119  -9.221  22.544  1.00 17.94
ATOM    379  N   GLY A  57      16.288  -7.645  19.216  1.00  6.84
ATOM    380  CA  GLY A  57      16.174  -7.019  17.841  1.00 16.15
ATOM    381  C   GLY A  57      14.740  -7.085  17.267  1.00 13.72
ATOM    382  O   GLY A  57      14.124  -8.016  17.752  1.00 12.70
ATOM    383  N   VAL A  58      14.068  -6.264  16.525  1.00 12.73
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ATOM    384  CA  VAL A  58      12.739  -6.308  16.070  1.00 11.16
ATOM    385  C   VAL A  58      12.715  -7.246  14.893  1.00 14.85
ATOM    386  O   VAL A  58      13.234  -6.891  13.849  1.00 18.64
ATOM    387  CB  VAL A  58      12.262  -4.984  15.352  1.00  6.54
ATOM    388  CG1 VAL A  58      10.894  -4.974  14.731  1.00  5.89
ATOM    389  CG2 VAL A  58      12.650  -3.840  16.331  1.00  5.86
ATOM    390  N   GLY A  59      12.209  -8.465  15.008  1.00 21.96
ATOM    391  CA  GLY A  59      12.120  -9.385  13.874  1.00 17.81
ATOM    392  C   GLY A  59      10.645  -9.561  13.550  1.00 23.35
ATOM    393  O   GLY A  59       9.919  -8.579  13.249  1.00 27.99
ATOM    394  N   GLY A  60      10.166 -10.805  13.623  1.00 18.75
ATOM    395  CA  GLY A  60       8.841 -11.142  13.285  1.00 11.46
ATOM    396  C   GLY A  60       8.550 -10.833  11.851  1.00 14.56
ATOM    397  O   GLY A  60       9.160 -11.439  11.003  1.00 16.32
ATOM    398  N   PRO A  61       7.505 -10.103  11.612  1.00 12.10
ATOM    399  CA  PRO A  61       7.123  -9.774  10.250  1.00 14.70
ATOM    400  C   PRO A  61       8.230  -8.941   9.570  1.00 22.17
ATOM    401  O   PRO A  61       8.143  -8.758   8.344  1.00 25.74
ATOM    402  CB  PRO A  61       5.911  -8.860  10.332  1.00 14.30
ATOM    403  CG  PRO A  61       5.880  -8.514  11.784  1.00 13.62
ATOM    404  CD  PRO A  61       6.723  -9.417  12.576  1.00 12.29
ATOM    405  N   TYR A  62       9.162  -8.257  10.292  1.00 21.56
ATOM    406  CA  TYR A  62       9.973  -7.242   9.674  1.00 17.07
ATOM    407  C   TYR A  62      11.133  -7.907   9.047  1.00 18.73
ATOM    408  O   TYR A  62      12.132  -8.213   9.691  1.00 22.39
ATOM    409  CB  TYR A  62      10.504  -6.401  10.803  1.00 17.51
ATOM    410  CG  TYR A  62      11.461  -5.421  10.236  1.00 15.23
ATOM    411  CD1 TYR A  62      11.343  -4.920   9.032  1.00 17.79
ATOM    412  CD2 TYR A  62      12.465  -4.971  10.969  1.00 19.09
ATOM    413  CE1 TYR A  62      12.206  -3.997   8.506  1.00 19.28
ATOM    414  CE2 TYR A  62      13.438  -4.101  10.490  1.00 25.40
ATOM    415  CZ  TYR A  62      13.327  -3.571   9.186  1.00 20.95
ATOM    416  OH  TYR A  62      14.320  -2.649   8.791  1.00 14.70
ATOM    417  N   ASP A  63      10.998  -8.419   7.816  1.00 19.47
ATOM    418  CA  ASP A  63      12.137  -9.011   7.081  1.00 17.52
ATOM    419  C   ASP A  63      13.027  -7.973   6.453  1.00 17.97
ATOM    420  O   ASP A  63      13.628  -8.442   5.512  1.00 14.94
ATOM    421  CB  ASP A  63      11.474  -9.873   6.015  1.00 17.16
ATOM    422  CG  ASP A  63      10.563  -9.136   5.096  1.00 27.75
ATOM    423  OD1 ASP A  63      10.049  -8.030   5.281  1.00 34.11
ATOM    424  OD2 ASP A  63      10.300  -9.635   4.002  1.00 44.13
ATOM    425  N   ALA A  64      13.089  -6.685   6.584  1.00 15.36
ATOM    426  CA  ALA A  64      14.054  -5.725   6.098  1.00 17.14
ATOM    427  C   ALA A  64      14.118  -5.780   4.589  1.00 21.10
ATOM    428  O   ALA A  64      15.193  -5.861   3.968  1.00 23.12
ATOM    429  CB  ALA A  64      15.458  -5.861   6.646  1.00 20.45
ATOM    430  N   ALA A  65      12.946  -6.009   4.006  1.00 22.21
ATOM    431  CA  ALA A  65      12.817  -6.072   2.565  1.00 21.81
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ATOM	432	C	ALA	A	65	13.143	-4.857	1.745	1.00	21.76
ATOM	433	O	ALA	A	65	12.855	-3.801	2.229	1.00	23.60
ATOM	434	CB	ALA	A	65	11.384	-6.390	2.364	1.00	17.31
ATOM	435	N	LEU	A	66	13.401	-4.866	0.402	1.00	21.48
ATOM	436	CA	LEU	A	66	13.763	-3.581	-0.216	1.00	13.20
ATOM	437	C	LEU	A	66	12.469	-2.913	-0.452	1.00	13.90
ATOM	438	O	LEU	A	66	12.548	-1.767	-0.197	1.00	11.85
ATOM	439	CB	LEU	A	66	14.593	-3.602	-1.470	1.00	3.92
ATOM	440	CG	LEU	A	66	15.891	-4.308	-1.191	1.00	9.05
ATOM	441	CD1	LEU	A	66	16.509	-4.725	-2.438	1.00	12.78
ATOM	442	CD2	LEU	A	66	16.569	-3.119	-0.580	1.00	13.44
ATOM	443	N	ALA	A	67	11.413	-3.625	-0.801	1.00	14.94
ATOM	444	CA	ALA	A	67	10.253	-2.759	-1.277	1.00	12.42
ATOM	445	C	ALA	A	67	9.626	-1.879	-0.224	1.00	14.21
ATOM	446	O	ALA	A	67	9.218	-0.818	-0.643	1.00	14.29
ATOM	447	CB	ALA	A	67	9.089	-3.588	-1.781	1.00	3.90
ATOM	448	N	THR	A	68	9.494	-2.409	1.006	1.00	12.11
ATOM	449	CA	THR	A	68	8.780	-1.647	1.997	1.00	11.77
ATOM	450	C	THR	A	68	9.242	-0.214	2.219	1.00	13.05
ATOM	451	O	THR	A	68	8.597	0.683	2.766	1.00	11.13
ATOM	452	CB	THR	A	68	8.892	-2.488	3.241	1.00	13.93
ATOM	453	OG1	THR	A	68	10.145	-3.150	3.224	1.00	27.44
ATOM	454	CG2	THR	A	68	7.783	-3.459	3.087	1.00	13.39
ATOM	455	N	ASN	A	69	10.450	-0.057	1.808	1.00	7.59
ATOM	456	CA	ASN	A	69	11.020	1.236	1.791	1.00	8.76
ATOM	457	C	ASN	A	69	10.095	2.165	1.047	1.00	10.28
ATOM	458	O	ASN	A	69	9.950	3.345	1.305	1.00	5.30
ATOM	459	CB	ASN	A	69	12.461	1.251	1.231	1.00	5.54
ATOM	460	CG	ASN	A	69	13.374	1.207	2.398	1.00	15.08
ATOM	461	OD1	ASN	A	69	13.307	2.124	3.275	1.00	31.90
ATOM	462	ND2	ASN	A	69	14.048	0.099	2.360	1.00	4.51
ATOM	463	N	PHE	A	70	9.390	1.656	0.079	1.00	19.09
ATOM	464	CA	PHE	A	70	8.552	2.619	-0.631	1.00	21.80
ATOM	465	C	PHE	A	70	7.157	2.836	-0.123	1.00	23.36
ATOM	466	O	PHE	A	70	6.509	3.717	-0.724	1.00	25.74
ATOM	467	CB	PHE	A	70	8.547	2.386	-2.082	1.00	17.38
ATOM	468	CG	PHE	A	70	9.870	2.360	-2.770	1.00	15.72
ATOM	469	CD1	PHE	A	70	10.080	3.430	-3.576	1.00	5.15
ATOM	470	CD2	PHE	A	70	10.702	1.245	-2.497	1.00	7.61
ATOM	471	CE1	PHE	A	70	11.268	3.330	-4.191	1.00	16.05
ATOM	472	CE2	PHE	A	70	11.913	1.267	-3.168	1.00	22.23
ATOM	473	CZ	PHE	A	70	12.199	2.314	-4.016	1.00	9.57
ATOM	474	N	LEU	A	71	6.765	2.246	1.034	1.00	25.53
ATOM	475	CA	LEU	A	71	5.506	2.725	1.599	1.00	24.24
ATOM	476	C	LEU	A	71	5.649	4.037	2.343	1.00	27.91
ATOM	477	O	LEU	A	71	6.694	4.521	2.750	1.00	28.86
ATOM	478	CB	LEU	A	71	5.150	1.635	2.535	1.00	19.99
ATOM	479	CG	LEU	A	71	5.003	0.342	1.873	1.00	16.09

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ATOM    480  CD1 LEU A  71       4.879  -0.764   2.885  1.00 18.12
ATOM    481  CD2 LEU A  71       3.786   0.546   1.000  1.00 18.24
ATOM    482  N   PRO A  72       4.535   4.663   2.529  1.00 33.01
ATOM    483  CA  PRO A  72       4.389   5.888   3.311  1.00 34.96
ATOM    484  C   PRO A  72       4.865   5.590   4.778  1.00 32.90
ATOM    485  O   PRO A  72       4.619   4.512   5.331  1.00 28.55
ATOM    486  CB  PRO A  72       2.983   6.453   3.095  1.00 32.98
ATOM    487  CG  PRO A  72       2.224   5.189   2.827  1.00 30.36
ATOM    488  CD  PRO A  72       3.188   4.093   2.380  1.00 33.56
ATOM    489  N   ARG A  73       5.601   6.610   5.221  1.00 27.54
ATOM    490  CA  ARG A  73       6.325   6.547   6.408  1.00 25.42
ATOM    491  C   ARG A  73       7.613   5.755   6.321  1.00 21.78
ATOM    492  O   ARG A  73       8.360   5.950   7.304  1.00 29.61
ATOM    493  CB  ARG A  73       5.469   5.978   7.549  1.00 24.29
ATOM    494  CG  ARG A  73       4.575   6.998   8.155  1.00 23.47
ATOM    495  CD  ARG A  73       3.818   6.793   9.360  1.00 29.73
ATOM    496  NE  ARG A  73       3.222   5.460   9.392  1.00 36.30
ATOM    497  CZ  ARG A  73       2.891   5.312  10.713  1.00 42.26
ATOM    498  NH1 ARG A  73       3.145   6.288  11.555  1.00 26.57
ATOM    499  NH2 ARG A  73       2.320   4.144  10.883  1.00 39.03
ATOM    500  N   GLY A  74       7.868   4.909   5.326  1.00  8.42
ATOM    501  CA  GLY A  74       9.120   4.291   5.332  1.00  5.06
ATOM    502  C   GLY A  74       9.243   2.858   5.508  1.00 12.74
ATOM    503  O   GLY A  74      10.256   2.286   5.317  1.00 16.46
ATOM    504  N   THR A  75       8.145   2.321   5.906  1.00 12.82
ATOM    505  CA  THR A  75       8.036   0.869   6.008  1.00 11.14
ATOM    506  C   THR A  75       6.625   0.428   6.134  1.00 10.64
ATOM    507  O   THR A  75       5.757   1.231   5.949  1.00  9.36
ATOM    508  CB  THR A  75       8.843   0.398   7.219  1.00  6.97
ATOM    509  OG1 THR A  75       8.938  -0.950   7.125  1.00  5.64
ATOM    510  CG2 THR A  75       8.108   0.865   8.603  1.00  6.30
ATOM    511  N   SER A  76       6.409  -0.858   6.259  1.00 10.07
ATOM    512  CA  SER A  76       5.061  -1.384   6.354  1.00 13.33
ATOM    513  C   SER A  76       4.405  -1.163   7.747  1.00 21.87
ATOM    514  O   SER A  76       5.228  -1.102   8.679  1.00 24.22
ATOM    515  CB  SER A  76       5.030  -2.832   6.083  1.00  4.81
ATOM    516  OG  SER A  76       5.327  -3.664   7.107  1.00 16.98
ATOM    517  N   GLN A  77       3.082  -1.100   7.911  1.00 24.90
ATOM    518  CA  GLN A  77       2.454  -1.020   9.166  1.00 23.85
ATOM    519  C   GLN A  77       2.643  -2.236  10.015  1.00 19.58
ATOM    520  O   GLN A  77       2.908  -2.140  11.203  1.00 15.15
ATOM    521  CB  GLN A  77       0.983  -0.703   9.217  1.00 32.64
ATOM    522  CG  GLN A  77       0.567  -0.580  10.642  1.00 49.56
ATOM    523  CD  GLN A  77       0.689   0.785  11.194  1.00 65.91
ATOM    524  OE1 GLN A  77       0.956   0.869  12.356  1.00 66.06
ATOM    525  NE2 GLN A  77       0.481   1.750  10.350  1.00 68.91
ATOM    526  N   ALA A  78       2.754  -3.376   9.402  1.00 15.90
ATOM    527  CA  ALA A  78       3.071  -4.577  10.073  1.00 19.47
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ATOM	528	C	ALA	A	78	4.381	-4.332	10.819	1.00	24.48
ATOM	529	O	ALA	A	78	4.389	-4.729	11.983	1.00	26.91
ATOM	530	CB	ALA	A	78	3.390	-5.808	9.336	1.00	17.23
ATOM	531	N	ASN	A	79	5.350	-3.863	10.093	1.00	21.58
ATOM	532	CA	ASN	A	79	6.602	-3.576	10.774	1.00	20.62
ATOM	533	C	ASN	A	79	6.480	-2.673	11.969	1.00	20.93
ATOM	534	O	ASN	A	79	6.975	-2.944	13.053	1.00	15.52
ATOM	535	CB	ASN	A	79	7.474	-3.069	9.670	1.00	24.79
ATOM	536	CG	ASN	A	79	7.933	-4.238	8.824	1.00	28.76
ATOM	537	OD1	ASN	A	79	7.867	-5.439	9.091	1.00	25.30
ATOM	538	ND2	ASN	A	79	8.488	-3.891	7.660	1.00	24.90
ATOM	539	N	ILE	A	80	5.731	-1.611	11.936	1.00	15.93
ATOM	540	CA	ILE	A	80	5.586	-0.574	12.924	1.00	17.00
ATOM	541	C	ILE	A	80	4.925	-1.187	14.118	1.00	20.63
ATOM	542	O	ILE	A	80	5.234	-0.939	15.264	1.00	18.79
ATOM	543	CB	ILE	A	80	4.756	0.629	12.436	1.00	11.98
ATOM	544	CG1	ILE	A	80	5.627	1.124	11.297	1.00	9.50
ATOM	545	CG2	ILE	A	80	4.379	1.728	13.354	1.00	16.27
ATOM	546	CD1	ILE	A	80	5.007	2.071	10.424	1.00	8.15
ATOM	547	N	ASP	A	81	4.017	-2.019	13.708	1.00	19.21
ATOM	548	CA	ASP	A	81	3.304	-2.778	14.728	1.00	15.15
ATOM	549	C	ASP	A	81	4.147	-3.711	15.510	1.00	15.77
ATOM	550	O	ASP	A	81	4.084	-3.697	16.695	1.00	15.82
ATOM	551	CB	ASP	A	81	2.291	-3.438	13.868	1.00	26.36
ATOM	552	CG	ASP	A	81	1.065	-2.530	13.790	1.00	23.71
ATOM	553	OD1	ASP	A	81	1.105	-1.355	14.226	1.00	14.33
ATOM	554	OD2	ASP	A	81	0.061	-3.125	13.222	1.00	33.05
ATOM	555	N	GLU	A	82	5.148	-4.447	15.096	1.00	16.07
ATOM	556	CA	GLU	A	82	5.984	-5.318	15.882	1.00	14.77
ATOM	557	C	GLU	A	82	6.839	-4.355	16.667	1.00	19.33
ATOM	558	O	GLU	A	82	7.315	-4.708	17.752	1.00	23.58
ATOM	559	CB	GLU	A	82	6.998	-6.031	15.064	1.00	13.20
ATOM	560	CG	GLU	A	82	7.792	-7.239	15.476	1.00	23.09
ATOM	561	CD	GLU	A	82	6.767	-8.114	16.185	1.00	29.68
ATOM	562	OE1	GLU	A	82	5.666	-7.670	16.403	1.00	26.63
ATOM	563	OE2	GLU	A	82	7.273	-9.181	16.411	1.00	33.08
ATOM	564	N	GLY	A	83	7.228	-3.227	16.199	1.00	16.79
ATOM	565	CA	GLY	A	83	8.033	-2.428	17.140	1.00	17.32
ATOM	566	C	GLY	A	83	7.238	-2.018	18.366	1.00	17.54
ATOM	567	O	GLY	A	83	7.561	-2.103	19.528	1.00	15.06
ATOM	568	N	LYS	A	84	6.093	-1.408	18.114	1.00	18.72
ATOM	569	CA	LYS	A	84	5.050	-1.146	19.096	1.00	16.90
ATOM	570	C	LYS	A	84	4.893	-2.337	20.057	1.00	17.74
ATOM	571	O	LYS	A	84	4.962	-2.265	21.295	1.00	14.31
ATOM	572	CB	LYS	A	84	3.799	-0.872	18.307	1.00	14.62
ATOM	573	CG	LYS	A	84	3.535	0.565	18.291	1.00	19.30
ATOM	574	CD	LYS	A	84	2.787	1.013	17.044	1.00	34.24
ATOM	575	CE	LYS	A	84	1.568	1.902	17.337	1.00	37.70

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ATOM    576  NZ  LYS A  84       0.346   1.226  16.827  1.00 48.42
ATOM    577  N   ARG A  85       4.617  -3.506  19.519  1.00 18.50
ATOM    578  CA  ARG A  85       4.583  -4.705  20.280  1.00 19.04
ATOM    579  C   ARG A  85       5.677  -4.733  21.308  1.00 19.63
ATOM    580  O   ARG A  85       5.442  -5.192  22.383  1.00 19.24
ATOM    581  CB  ARG A  85       4.740  -5.979  19.464  1.00 14.74
ATOM    582  CG  ARG A  85       3.843  -7.094  19.887  1.00  8.85
ATOM    583  CD  ARG A  85       4.146  -8.554  19.705  1.00  7.20
ATOM    584  NE  ARG A  85       5.483  -8.898  19.194  1.00 20.30
ATOM    585  CZ  ARG A  85       6.170  -9.705  19.899  1.00 18.19
ATOM    586  NH1 ARG A  85       5.627 -10.161  21.040  1.00 34.03
ATOM    587  NH2 ARG A  85       7.345  -9.979  19.555  1.00 15.36
ATOM    588  N   LEU A  86       6.901  -4.586  20.956  1.00 22.21
ATOM    589  CA  LEU A  86       8.006  -4.792  21.873  1.00 20.94
ATOM    590  C   LEU A  86       8.044  -3.637  22.803  1.00 20.73
ATOM    591  O   LEU A  86       8.155  -3.970  23.925  1.00 22.18
ATOM    592  CB  LEU A  86       9.333  -4.932  21.168  1.00  6.67
ATOM    593  CG  LEU A  86       9.358  -6.241  20.282  1.00 11.45
ATOM    594  CD1 LEU A  86      10.546  -6.054  19.287  1.00 18.60
ATOM    595  CD2 LEU A  86       9.362  -7.516  21.020  1.00  5.17
ATOM    596  N   PHE A  87       7.700  -2.446  22.529  1.00 16.79
ATOM    597  CA  PHE A  87       7.850  -1.416  23.492  1.00 18.21
ATOM    598  C   PHE A  87       6.939  -1.805  24.618  1.00 26.51
ATOM    599  O   PHE A  87       7.082  -1.565  25.839  1.00 30.36
ATOM    600  CB  PHE A  87       7.498  -0.118  22.846  1.00 15.81
ATOM    601  CG  PHE A  87       8.661   0.503  22.128  1.00 22.72
ATOM    602  CD1 PHE A  87       9.625   1.163  22.795  1.00 25.90
ATOM    603  CD2 PHE A  87       8.800   0.446  20.774  1.00 24.19
ATOM    604  CE1 PHE A  87      10.699   1.781  22.220  1.00 26.46
ATOM    605  CE2 PHE A  87       9.871   0.991  20.153  1.00 29.24
ATOM    606  CZ  PHE A  87      10.827   1.669  20.849  1.00 20.81
ATOM    607  N   ALA A  88       5.862  -2.422  24.266  1.00 29.15
ATOM    608  CA  ALA A  88       4.772  -2.699  25.195  1.00 22.92
ATOM    609  C   ALA A  88       5.186  -3.837  26.068  1.00 22.03
ATOM    610  O   ALA A  88       4.974  -3.879  27.284  1.00 27.02
ATOM    611  CB  ALA A  88       3.551  -2.803  24.299  1.00 22.13
ATOM    612  N   LEU A  89       5.649  -4.897  25.531  1.00 19.16
ATOM    613  CA  LEU A  89       6.188  -6.032  26.208  1.00 19.29
ATOM    614  C   LEU A  89       7.250  -5.507  27.133  1.00 22.06
ATOM    615  O   LEU A  89       7.449  -6.050  28.177  1.00 20.49
ATOM    616  CB  LEU A  89       7.021  -6.863  25.221  1.00 18.41
ATOM    617  CG  LEU A  89       7.477  -8.167  25.834  1.00 20.45
ATOM    618  CD1 LEU A  89       6.326  -8.707  26.627  1.00 17.22
ATOM    619  CD2 LEU A  89       8.060  -9.057  24.769  1.00 18.83
ATOM    620  N   ALA A  90       8.124  -4.644  26.722  1.00 22.80
ATOM    621  CA  ALA A  90       9.027  -4.137  27.701  1.00 24.14
ATOM    622  C   ALA A  90       8.237  -3.488  28.849  1.00 23.63
ATOM    623  O   ALA A  90       8.414  -3.835  30.071  1.00 22.73
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ATOM	624	CB	ALA	A	90	10.080	-3.253	27.139	1.00	7.74
ATOM	625	N	ASN	A	91	7.457	-2.445	28.732	1.00	25.45
ATOM	626	CA	ASN	A	91	6.665	-1.979	29.870	1.00	27.25
ATOM	627	C	ASN	A	91	5.847	-2.996	30.656	1.00	30.97
ATOM	628	O	ASN	A	91	5.346	-2.884	31.768	1.00	27.64
ATOM	629	CB	ASN	A	91	5.560	-1.206	29.125	1.00	29.14
ATOM	630	CG	ASN	A	91	4.946	-0.345	30.216	1.00	31.73
ATOM	631	OD1	ASN	A	91	3.845	-0.692	30.645	1.00	46.76
ATOM	632	ND2	ASN	A	91	5.641	0.629	30.643	1.00	29.03
ATOM	633	N	GLN	A	92	5.369	-4.008	29.969	1.00	35.37
ATOM	634	CA	GLN	A	92	4.702	-5.141	30.591	1.00	35.55
ATOM	635	C	GLN	A	92	5.619	-6.072	31.352	1.00	34.28
ATOM	636	O	GLN	A	92	5.227	-6.519	32.440	1.00	39.47
ATOM	637	CB	GLN	A	92	3.866	-5.903	29.573	1.00	54.94
ATOM	638	CG	GLN	A	92	2.689	-6.698	30.142	1.00	78.63
ATOM	639	CD	GLN	A	92	2.806	-8.167	29.805	1.00	93.87
ATOM	640	OE1	GLN	A	92	3.597	-8.840	30.475	1.00	96.99
ATOM	641	NE2	GLN	A	92	2.083	-8.696	28.824	1.00	97.81
ATOM	642	N	LYS	A	93	6.859	-6.403	31.050	1.00	31.97
ATOM	643	CA	LYS	A	93	7.675	-7.204	31.972	1.00	25.22
ATOM	644	C	LYS	A	93	8.381	-6.298	33.015	1.00	24.68
ATOM	645	O	LYS	A	93	8.716	-6.793	34.075	1.00	32.13
ATOM	646	CB	LYS	A	93	8.673	-7.980	31.148	1.00	10.86
ATOM	647	CG	LYS	A	93	8.225	-8.963	30.159	1.00	24.26
ATOM	648	CD	LYS	A	93	9.362	-9.966	29.986	1.00	21.96
ATOM	649	CE	LYS	A	93	9.093	-10.718	28.658	1.00	23.78
ATOM	650	NZ	LYS	A	93	10.084	-11.805	28.300	1.00	25.87
ATOM	651	N	CYS	A	94	8.752	-5.096	32.774	1.00	16.62
ATOM	652	CA	CYS	A	94	9.752	-4.412	33.480	1.00	18.95
ATOM	653	C	CYS	A	94	9.512	-2.936	33.537	1.00	24.83
ATOM	654	O	CYS	A	94	10.184	-2.017	33.150	1.00	26.80
ATOM	655	CB	CYS	A	94	11.147	-4.691	32.911	1.00	3.14
ATOM	656	SG	CYS	A	94	11.618	-6.437	32.882	1.00	25.28
ATOM	657	N	PRO	A	95	8.403	-2.561	34.086	1.00	26.08
ATOM	658	CA	PRO	A	95	7.891	-1.202	33.878	1.00	26.11
ATOM	659	C	PRO	A	95	8.960	-0.259	34.299	1.00	27.32
ATOM	660	O	PRO	A	95	8.776	0.966	34.108	1.00	29.08
ATOM	661	CB	PRO	A	95	6.609	-1.090	34.747	1.00	20.75
ATOM	662	CG	PRO	A	95	6.587	-2.421	35.322	1.00	19.04
ATOM	663	CD	PRO	A	95	7.363	-3.461	34.509	1.00	22.55
ATOM	664	N	ASN	A	96	9.836	-0.776	35.193	1.00	31.44
ATOM	665	CA	ASN	A	96	10.559	0.274	35.966	1.00	35.38
ATOM	666	C	ASN	A	96	11.891	0.476	35.353	1.00	33.83
ATOM	667	O	ASN	A	96	12.599	1.359	35.684	1.00	33.31
ATOM	668	CB	ASN	A	96	10.558	-0.099	37.429	1.00	53.70
ATOM	669	CG	ASN	A	96	9.238	0.342	38.026	1.00	61.69
ATOM	670	OD1	ASN	A	96	8.758	1.432	37.706	1.00	64.33
ATOM	671	ND2	ASN	A	96	8.676	-0.526	38.861	1.00	67.25

ATOM	672	N	THR	A	97	12.287	-0.409	34.507	1.00	30.32
ATOM	673	CA	THR	A	97	13.519	-0.367	33.794	1.00	22.83
ATOM	674	C	THR	A	97	13.404	0.493	32.534	1.00	22.44
ATOM	675	O	THR	A	97	12.446	0.779	31.816	1.00	21.14
ATOM	676	CB	THR	A	97	13.835	-1.851	33.705	1.00	25.87
ATOM	677	OG1	THR	A	97	14.602	-1.915	32.528	1.00	38.91
ATOM	678	CG2	THR	A	97	12.769	-2.901	33.621	1.00	24.22
ATOM	679	N	PRO	A	98	14.393	1.415	32.408	1.00	20.59
ATOM	680	CA	PRO	A	98	14.513	2.292	31.254	1.00	18.15
ATOM	681	C	PRO	A	98	14.882	1.494	29.978	1.00	16.07
ATOM	682	O	PRO	A	98	15.622	0.462	29.934	1.00	17.19
ATOM	683	CB	PRO	A	98	15.563	3.339	31.676	1.00	14.55
ATOM	684	CG	PRO	A	98	16.270	2.646	32.699	1.00	12.29
ATOM	685	CD	PRO	A	98	15.735	1.331	33.046	1.00	12.02
ATOM	686	N	VAL	A	99	14.322	2.107	28.940	1.00	13.81
ATOM	687	CA	VAL	A	99	14.225	1.544	27.632	1.00	14.02
ATOM	688	C	VAL	A	99	14.956	2.407	26.663	1.00	10.66
ATOM	689	O	VAL	A	99	14.716	3.679	26.712	1.00	6.90
ATOM	690	CB	VAL	A	99	12.673	1.343	27.335	1.00	2.87
ATOM	691	CG1	VAL	A	99	12.666	1.272	25.872	1.00	17.40
ATOM	692	CG2	VAL	A	99	12.442	-0.111	27.744	1.00	5.75
ATOM	693	N	VAL	A	100	15.885	1.776	25.861	1.00	6.45
ATOM	694	CA	VAL	A	100	16.525	2.755	24.900	1.00	9.61
ATOM	695	C	VAL	A	100	16.389	2.159	23.561	1.00	10.79
ATOM	696	O	VAL	A	100	16.256	0.973	23.477	1.00	9.11
ATOM	697	CB	VAL	A	100	17.877	3.260	25.197	1.00	8.05
ATOM	698	CG1	VAL	A	100	17.824	4.252	26.336	1.00	6.05
ATOM	699	CG2	VAL	A	100	18.853	2.053	25.591	1.00	6.68
ATOM	700	N	ALA	A	101	16.277	2.928	22.511	1.00	13.14
ATOM	701	CA	ALA	A	101	16.127	2.266	21.183	1.00	15.67
ATOM	702	C	ALA	A	101	17.065	2.747	20.053	1.00	12.08
ATOM	703	O	ALA	A	101	17.261	4.042	19.907	1.00	11.16
ATOM	704	CB	ALA	A	101	14.685	2.609	20.812	1.00	6.57
ATOM	705	N	GLY	A	102	17.218	1.787	19.099	1.00	7.53
ATOM	706	CA	GLY	A	102	17.949	2.415	17.939	1.00	7.10
ATOM	707	C	GLY	A	102	17.477	1.803	16.744	1.00	7.27
ATOM	708	O	GLY	A	102	17.102	0.621	16.878	1.00	10.83
ATOM	709	N	GLY	A	103	17.706	2.407	15.648	1.00	7.80
ATOM	710	CA	GLY	A	103	17.446	1.745	14.356	1.00	5.33
ATOM	711	C	GLY	A	103	18.303	2.211	13.180	1.00	7.56
ATOM	712	O	GLY	A	103	18.785	3.340	13.227	1.00	6.88
ATOM	713	N	TYR	A	104	18.490	1.387	12.139	1.00	7.09
ATOM	714	CA	TYR	A	104	19.392	1.682	11.069	1.00	5.99
ATOM	715	C	TYR	A	104	18.705	1.614	9.705	1.00	9.47
ATOM	716	O	TYR	A	104	18.115	0.638	9.441	1.00	6.46
ATOM	717	CB	TYR	A	104	20.592	0.797	11.079	1.00	5.40
ATOM	718	CG	TYR	A	104	21.436	1.078	9.876	1.00	8.05
ATOM	719	CD1	TYR	A	104	21.708	2.302	9.352	1.00	5.91

ATOM	720	CD2	TYR	A	104	21.961	-0.044	9.172	1.00	6.85
ATOM	721	CE1	TYR	A	104	22.447	2.513	8.186	1.00	5.61
ATOM	722	CE2	TYR	A	104	22.751	0.052	8.072	1.00	7.49
ATOM	723	CZ	TYR	A	104	22.972	1.377	7.608	1.00	11.08
ATOM	724	OH	TYR	A	104	23.795	1.509	6.479	1.00	14.32
ATOM	725	N	SER	A	105	18.939	2.975	8.852	1.00	18.39
ATOM	726	CA	SER	A	105	18.190	2.854	7.601	1.00	9.66
ATOM	727	C	SER	A	105	16.763	2.370	7.722	1.00	6.10
ATOM	728	O	SER	A	105	16.090	3.304	8.077	1.00	5.63
ATOM	729	CB	SER	A	105	19.124	2.159	6.607	1.00	8.55
ATOM	730	OG	SER	A	105	18.553	1.685	5.463	1.00	24.30
ATOM	731	N	GLN	A	106	16.241	1.405	7.079	1.00	9.93
ATOM	732	CA	GLN	A	106	14.759	1.316	7.002	1.00	8.25
ATOM	733	C	GLN	A	106	14.453	1.089	8.473	1.00	8.51
ATOM	734	O	GLN	A	106	13.470	1.683	8.862	1.00	6.31
ATOM	735	CB	GLN	A	106	14.239	0.393	5.940	1.00	7.45
ATOM	736	CG	GLN	A	106	13.184	-0.528	6.465	1.00	18.04
ATOM	737	CD	GLN	A	106	12.228	-1.220	5.581	1.00	16.87
ATOM	738	OE1	GLN	A	106	11.024	-1.180	5.492	1.00	17.59
ATOM	739	NE2	GLN	A	106	12.643	-2.032	4.713	1.00	8.32
ATOM	740	N	GLY	A	107	15.269	0.310	9.172	1.00	7.13
ATOM	741	CA	GLY	A	107	15.190	0.159	10.606	1.00	4.61
ATOM	742	C	GLY	A	107	15.048	1.472	11.356	1.00	8.27
ATOM	743	O	GLY	A	107	14.219	1.511	12.290	1.00	6.52
ATOM	744	N	ALA	A	108	15.653	2.637	11.033	1.00	6.44
ATOM	745	CA	ALA	A	108	15.266	3.864	11.641	1.00	7.41
ATOM	746	C	ALA	A	108	13.813	4.346	11.471	1.00	11.76
ATOM	747	O	ALA	A	108	13.150	4.914	12.298	1.00	12.64
ATOM	748	CB	ALA	A	108	16.121	5.006	11.170	1.00	13.93
ATOM	749	N	ALA	A	109	13.321	4.312	10.267	1.00	9.78
ATOM	750	CA	ALA	A	109	12.056	4.685	9.861	1.00	10.47
ATOM	751	C	ALA	A	109	11.093	3.858	10.727	1.00	12.32
ATOM	752	O	ALA	A	109	10.016	4.391	11.035	1.00	14.67
ATOM	753	CB	ALA	A	109	12.035	4.173	8.456	1.00	10.24
ATOM	754	N	LEU	A	110	11.259	2.690	11.077	1.00	4.34
ATOM	755	CA	LEU	A	110	10.458	1.760	11.783	1.00	11.71
ATOM	756	C	LEU	A	110	10.305	2.253	13.203	1.00	15.26
ATOM	757	O	LEU	A	110	9.298	2.672	13.685	1.00	18.07
ATOM	758	CB	LEU	A	110	11.031	0.319	11.634	1.00	7.52
ATOM	759	CG	LEU	A	110	10.247	-0.801	12.258	1.00	8.41
ATOM	760	CD1	LEU	A	110	10.685	-2.233	11.862	1.00	7.17
ATOM	761	CD2	LEU	A	110	10.278	-0.659	13.783	1.00	5.25
ATOM	762	N	ILE	A	111	11.397	2.373	13.907	1.00	15.77
ATOM	763	CA	ILE	A	111	11.510	2.860	15.246	1.00	12.22
ATOM	764	C	ILE	A	111	11.027	4.255	15.234	1.00	9.39
ATOM	765	O	ILE	A	111	10.404	4.636	16.241	1.00	12.54
ATOM	766	CB	ILE	A	111	12.977	2.814	15.685	1.00	15.55
ATOM	767	CG1	ILE	A	111	13.222	1.279	15.805	1.00	14.19

ATOM	768	CG2	ILE	A	111	13.195	3.465	17.005	1.00	4.64
ATOM	769	CD1	ILE	A	111	12.410	0.887	17.002	1.00	14.88
ATOM	770	N	ALA	A	112	11.309	5.170	14.341	1.00	11.00
ATOM	771	CA	ALA	A	112	10.792	6.528	14.427	1.00	12.45
ATOM	772	C	ALA	A	112	9.266	6.455	14.308	1.00	15.59
ATOM	773	O	ALA	A	112	8.728	7.131	15.154	1.00	18.13
ATOM	774	CB	ALA	A	112	11.334	7.505	13.486	1.00	5.70
ATOM	775	N	ALA	A	113	8.575	5.572	13.587	1.00	12.85
ATOM	776	CA	ALA	A	113	7.167	5.512	13.557	1.00	15.39
ATOM	777	C	ALA	A	113	6.475	5.093	14.861	1.00	18.21
ATOM	778	O	ALA	A	113	5.498	5.750	15.226	1.00	14.59
ATOM	779	CB	ALA	A	113	6.678	4.562	12.500	1.00	17.63
ATOM	780	N	ALA	A	114	6.937	3.948	15.303	1.00	16.02
ATOM	781	CA	ALA	A	114	6.483	3.218	16.412	1.00	16.43
ATOM	782	C	ALA	A	114	6.578	4.114	17.643	1.00	22.20
ATOM	783	O	ALA	A	114	5.673	4.321	18.426	1.00	18.94
ATOM	784	CB	ALA	A	114	7.474	2.084	16.565	1.00	4.69
ATOM	785	N	VAL	A	115	7.722	4.836	17.744	1.00	22.46
ATOM	786	CA	VAL	A	115	7.855	5.499	19.064	1.00	20.88
ATOM	787	C	VAL	A	115	6.670	6.469	19.007	1.00	22.71
ATOM	788	O	VAL	A	115	6.136	6.761	20.057	1.00	22.05
ATOM	789	CB	VAL	A	115	9.279	6.090	19.137	1.00	19.61
ATOM	790	CG1	VAL	A	115	9.396	7.259	20.122	1.00	8.35
ATOM	791	CG2	VAL	A	115	10.245	5.016	19.562	1.00	13.91
ATOM	792	N	SER	A	116	6.467	7.085	17.828	1.00	23.59
ATOM	793	CA	SER	A	116	5.539	8.172	17.736	1.00	23.68
ATOM	794	C	SER	A	116	4.169	7.647	18.120	1.00	23.77
ATOM	795	O	SER	A	116	3.333	8.523	18.399	1.00	27.35
ATOM	796	CB	SER	A	116	5.522	8.865	16.376	1.00	25.21
ATOM	797	OG	SER	A	116	5.168	8.043	15.277	1.00	28.05
ATOM	798	N	GLU	A	117	3.859	6.397	18.004	1.00	18.83
ATOM	799	CA	GLU	A	117	2.491	6.020	18.238	1.00	22.21
ATOM	800	C	GLU	A	117	2.461	5.474	19.653	1.00	30.46
ATOM	801	O	GLU	A	117	1.487	4.773	19.863	1.00	35.72
ATOM	802	CB	GLU	A	117	1.977	4.902	17.343	1.00	21.63
ATOM	803	CG	GLU	A	117	2.167	5.219	15.897	1.00	26.41
ATOM	804	CD	GLU	A	117	1.560	4.424	14.814	1.00	34.01
ATOM	805	OE1	GLU	A	117	0.912	3.440	15.046	1.00	32.59
ATOM	806	OE2	GLU	A	117	1.750	4.833	13.659	1.00	44.62
ATOM	807	N	LEU	A	118	3.438	5.570	20.512	1.00	34.45
ATOM	808	CA	LEU	A	118	3.326	5.006	21.812	1.00	33.64
ATOM	809	C	LEU	A	118	2.681	6.110	22.633	1.00	41.75
ATOM	810	O	LEU	A	118	2.594	7.267	22.370	1.00	39.90
ATOM	811	CB	LEU	A	118	4.600	4.668	22.392	1.00	29.44
ATOM	812	CG	LEU	A	118	5.628	3.891	21.645	1.00	26.36
ATOM	813	CD1	LEU	A	118	6.921	3.840	22.379	1.00	27.53
ATOM	814	CD2	LEU	A	118	5.110	2.520	21.536	1.00	20.69
ATOM	815	N	SER	A	119	2.076	5.794	23.726	1.00	48.86

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ATOM    816  CA  SER A 119       0.910   5.647  24.476  1.00 52.44
ATOM    817  C   SER A 119       1.212   6.063  25.866  1.00 52.57
ATOM    818  O   SER A 119       1.485   5.258  26.735  1.00 55.54
ATOM    819  CB  SER A 119       0.550   4.132  24.488  1.00 70.55
ATOM    820  OG  SER A 119       1.393   3.091  23.908  1.00 66.80
ATOM    821  N   GLY A 120       1.532   7.307  26.024  1.00 52.95
ATOM    822  CA  GLY A 120       1.910   7.761  27.382  1.00 53.35
ATOM    823  C   GLY A 120       2.944   7.109  28.291  1.00 49.09
ATOM    824  O   GLY A 120       4.086   7.617  28.358  1.00 49.66
ATOM    825  N   ALA A 121       2.526   6.129  29.102  1.00 42.97
ATOM    826  CA  ALA A 121       3.477   5.574  30.022  1.00 40.72
ATOM    827  C   ALA A 121       4.587   4.772  29.326  1.00 44.20
ATOM    828  O   ALA A 121       5.749   4.803  29.711  1.00 45.42
ATOM    829  CB  ALA A 121       2.965   4.542  30.903  1.00 36.34
ATOM    830  N   VAL A 122       4.122   4.035  28.312  1.00 41.15
ATOM    831  CA  VAL A 122       5.090   3.269  27.548  1.00 33.41
ATOM    832  C   VAL A 122       5.870   4.168  26.652  1.00 28.48
ATOM    833  O   VAL A 122       7.084   4.019  26.872  1.00 27.69
ATOM    834  CB  VAL A 122       4.424   2.056  26.952  1.00 30.22
ATOM    835  CG1 VAL A 122       2.924   1.997  27.098  1.00 28.03
ATOM    836  CG2 VAL A 122       4.891   1.836  25.551  1.00 23.22
ATOM    837  N   LYS A 123       5.424   5.310  26.177  1.00 23.16
ATOM    838  CA  LYS A 123       6.354   6.314  25.661  1.00 23.11
ATOM    839  C   LYS A 123       7.403   6.783  26.661  1.00 25.28
ATOM    840  O   LYS A 123       8.524   7.224  26.449  1.00 29.01
ATOM    841  CB  LYS A 123       5.561   7.502  25.100  1.00 23.54
ATOM    842  CG  LYS A 123       6.171   8.573  24.277  1.00 26.71
ATOM    843  CD  LYS A 123       5.400   9.775  23.888  1.00 43.07
ATOM    844  CE  LYS A 123       4.953   9.783  22.461  1.00 59.59
ATOM    845  NZ  LYS A 123       3.518   9.637  22.099  1.00 67.50
ATOM    846  N   GLU A 124       6.977   6.991  27.918  1.00 27.95
ATOM    847  CA  GLU A 124       7.845   7.700  28.863  1.00 27.29
ATOM    848  C   GLU A 124       8.910   6.706  29.243  1.00 25.21
ATOM    849  O   GLU A 124       9.993   7.165  29.769  1.00 21.21
ATOM    850  CB  GLU A 124       6.986   8.351  29.927  1.00 40.13
ATOM    851  CG  GLU A 124       7.588   8.609  31.295  1.00 57.40
ATOM    852  CD  GLU A 124       8.530   9.814  31.247  1.00 66.99
ATOM    853  OE1 GLU A 124       9.619   9.751  31.902  1.00 70.44
ATOM    854  OE2 GLU A 124       7.949  10.652  30.502  1.00 73.84
ATOM    855  N   GLN A 125       8.656   5.393  29.058  1.00 19.93
ATOM    856  CA  GLN A 125       9.761   4.509  29.546  1.00 17.98
ATOM    857  C   GLN A 125      10.865   4.556  28.521  1.00 24.28
ATOM    858  O   GLN A 125      11.964   4.107  28.815  1.00 21.47
ATOM    859  CB  GLN A 125       9.225   3.178  29.844  1.00  9.13
ATOM    860  CG  GLN A 125       9.901   2.001  30.299  1.00  9.05
ATOM    861  CD  GLN A 125       9.211   0.719  30.129  1.00 19.33
ATOM    862  OE1 GLN A 125       8.190   0.703  29.466  1.00 28.52
ATOM    863  NE2 GLN A 125       9.662  -0.396  30.684  1.00 13.34
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ATOM	864	N	VAL	A	126	10.593	5.188	27.319	1.00 25.30
ATOM	865	CA	VAL	A	126	11.738	5.124	26.361	1.00 22.55
ATOM	866	C	VAL	A	126	12.546	6.334	26.614	1.00 17.55
ATOM	867	O	VAL	A	126	12.109	7.408	26.329	1.00 12.79
ATOM	868	CB	VAL	A	126	11.227	4.560	25.022	1.00 23.76
ATOM	869	CG1	VAL	A	126	9.706	4.686	24.946	1.00 23.77
ATOM	870	CG2	VAL	A	126	11.795	5.081	23.743	1.00 23.81
ATOM	871	N	LYS	A	127	13.726	6.233	27.264	1.00 16.41
ATOM	872	CA	LYS	A	127	14.462	7.494	27.639	1.00 18.18
ATOM	873	C	LYS	A	127	15.239	8.063	26.488	1.00 18.49
ATOM	874	O	LYS	A	127	15.812	9.103	26.680	1.00 18.99
ATOM	875	CB	LYS	A	127	15.401	7.148	28.792	1.00 20.81
ATOM	876	CG	LYS	A	127	14.770	6.110	29.713	1.00 21.99
ATOM	877	CD	LYS	A	127	13.435	6.726	30.064	1.00 33.86
ATOM	878	CE	LYS	A	127	12.779	6.612	31.399	1.00 32.17
ATOM	879	NZ	LYS	A	127	12.279	7.863	31.993	1.00 45.34
ATOM	880	N	GLY	A	128	15.522	7.281	25.416	1.00 20.56
ATOM	881	CA	GLY	A	128	16.280	7.948	24.306	1.00 20.72
ATOM	882	C	GLY	A	128	16.358	7.104	23.063	1.00 17.71
ATOM	883	O	GLY	A	128	16.168	5.901	23.226	1.00 16.66
ATOM	884	N	VAL	A	129	16.451	7.725	21.892	1.00 16.16
ATOM	885	CA	VAL	A	129	16.497	6.872	20.691	1.00 13.82
ATOM	886	C	VAL	A	129	17.519	7.371	19.719	1.00 8.35
ATOM	887	O	VAL	A	129	17.602	8.553	19.556	1.00 3.85
ATOM	888	CB	VAL	A	129	15.192	6.426	20.054	1.00 11.02
ATOM	889	CG1	VAL	A	129	14.007	7.041	20.726	1.00 6.50
ATOM	890	CG2	VAL	A	129	15.051	6.729	18.571	1.00 10.03
ATOM	891	N	ALA	A	130	18.455	6.398	19.363	1.00 8.05
ATOM	892	CA	ALA	A	130	19.430	6.845	18.344	1.00 7.55
ATOM	893	C	ALA	A	130	19.078	6.293	16.958	1.00 11.17
ATOM	894	O	ALA	A	130	18.755	5.145	16.849	1.00 15.74
ATOM	895	CB	ALA	A	130	20.781	6.391	18.603	1.00 5.89
ATOM	896	N	LEU	A	131	18.911	6.953	15.892	1.00 7.36
ATOM	897	CA	LEU	A	131	18.635	6.625	14.553	1.00 7.70
ATOM	898	C	LEU	A	131	19.876	6.908	13.661	1.00 12.02
ATOM	899	O	LEU	A	131	20.436	8.033	13.604	1.00 6.80
ATOM	900	CB	LEU	A	131	17.604	7.713	14.102	1.00 8.40
ATOM	901	CG	LEU	A	131	16.160	7.830	14.575	1.00 6.67
ATOM	902	CD1	LEU	A	131	15.391	8.957	13.981	1.00 4.49
ATOM	903	CD2	LEU	A	131	15.481	6.488	14.324	1.00 5.12
ATOM	904	N	PHE	A	132	20.271	6.009	12.802	1.00 11.56
ATOM	905	CA	PHE	A	132	21.422	6.183	11.908	1.00 10.44
ATOM	906	C	PHE	A	132	20.965	6.013	10.478	1.00 8.46
ATOM	907	O	PHE	A	132	20.175	5.101	10.097	1.00 11.04
ATOM	908	CB	PHE	A	132	22.217	4.931	12.282	1.00 10.56
ATOM	909	CG	PHE	A	132	22.693	4.830	13.714	1.00 16.38
ATOM	910	CD1	PHE	A	132	21.951	4.029	14.542	1.00 13.36
ATOM	911	CD2	PHE	A	132	23.860	5.489	14.213	1.00 15.12

ATOM	912	CE1	PHE	A	132	22.342	3.911	15.889	1.00	14.91
ATOM	913	CE2	PHE	A	132	24.176	5.323	15.513	1.00	18.02
ATOM	914	CZ	PHE	A	132	23.426	4.530	16.403	1.00	15.09
ATOM	915	N	GLY	A	133	21.431	6.876	9.580	1.00	7.35
ATOM	916	CA	GLY	A	133	21.026	6.893	8.148	1.00	5.86
ATOM	917	C	GLY	A	133	19.503	6.919	8.061	1.00	12.25
ATOM	918	O	GLY	A	133	18.890	5.926	7.593	1.00	9.03
ATOM	919	N	TYR	A	134	18.926	8.070	8.532	1.00	9.85
ATOM	920	CA	TYR	A	134	17.455	8.022	8.838	1.00	7.40
ATOM	921	C	TYR	A	134	16.647	8.365	7.584	1.00	10.61
ATOM	922	O	TYR	A	134	16.785	9.513	7.131	1.00	5.85
ATOM	923	CB	TYR	A	134	17.161	9.128	9.836	1.00	7.27
ATOM	924	CG	TYR	A	134	15.842	9.393	10.391	1.00	7.89
ATOM	925	CD1	TYR	A	134	14.889	8.437	10.312	1.00	6.65
ATOM	926	CD2	TYR	A	134	15.661	10.651	10.948	1.00	11.44
ATOM	927	CE1	TYR	A	134	13.657	8.690	10.821	1.00	9.05
ATOM	928	CE2	TYR	A	134	14.408	10.928	11.467	1.00	12.89
ATOM	929	CZ	TYR	A	134	13.428	9.923	11.423	1.00	14.22
ATOM	930	OH	TYR	A	134	12.146	10.110	11.975	1.00	12.41
ATOM	931	N	THR	A	135	15.811	7.398	7.139	1.00	11.51
ATOM	932	CA	THR	A	135	15.229	7.581	5.789	1.00	7.71
ATOM	933	C	THR	A	135	14.082	8.530	5.825	1.00	10.36
ATOM	934	O	THR	A	135	13.845	8.878	4.727	1.00	11.26
ATOM	935	CB	THR	A	135	14.772	6.394	4.967	1.00	12.02
ATOM	936	OG1	THR	A	135	13.821	5.399	5.398	1.00	22.81
ATOM	937	CG2	THR	A	135	15.828	5.332	4.712	1.00	14.88
ATOM	938	N	GLN	A	136	13.632	9.105	6.928	1.00	15.28
ATOM	939	CA	GLN	A	136	12.596	10.134	6.968	1.00	16.48
ATOM	940	C	GLN	A	136	13.102	11.418	7.646	1.00	17.46
ATOM	941	O	GLN	A	136	12.292	12.231	8.035	1.00	12.82
ATOM	942	CB	GLN	A	136	11.336	9.671	7.701	1.00	5.71
ATOM	943	CG	GLN	A	136	11.178	8.191	7.263	1.00	13.60
ATOM	944	CD	GLN	A	136	10.504	8.264	5.932	1.00	14.65
ATOM	945	OE1	GLN	A	136	9.587	9.102	5.986	1.00	23.99
ATOM	946	NE2	GLN	A	136	10.852	7.529	4.914	1.00	14.68
ATOM	947	N	ASN	A	137	14.421	11.532	7.566	1.00	18.52
ATOM	948	CA	ASN	A	137	14.953	12.752	8.141	1.00	18.16
ATOM	949	C	ASN	A	137	14.301	13.929	7.458	1.00	19.79
ATOM	950	O	ASN	A	137	13.895	14.802	8.157	1.00	12.28
ATOM	951	CB	ASN	A	137	16.481	12.573	8.239	1.00	14.17
ATOM	952	CG	ASN	A	137	17.247	13.740	8.812	1.00	19.75
ATOM	953	OD1	ASN	A	137	17.821	14.341	7.934	1.00	14.52
ATOM	954	ND2	ASN	A	137	17.390	14.130	10.042	1.00	17.43
ATOM	955	N	LEU	A	138	14.180	14.062	6.141	1.00	27.31
ATOM	956	CA	LEU	A	138	13.640	15.270	5.553	1.00	25.53
ATOM	957	C	LEU	A	138	12.190	15.332	5.971	1.00	22.45
ATOM	958	O	LEU	A	138	11.710	16.281	6.549	1.00	25.13
ATOM	959	CB	LEU	A	138	13.632	15.269	4.056	1.00	41.28

ATOM	960	CG	LEU	A	138	13.713	16.582	3.303	1.00	31.76
ATOM	961	CD1	LEU	A	138	14.641	17.503	4.012	1.00	51.09
ATOM	962	CD2	LEU	A	138	14.207	16.573	1.958	1.00	46.20
ATOM	963	N	GLN	A	139	11.378	14.403	5.569	1.00	20.48
ATOM	964	CA	GLN	A	139	10.034	14.390	6.037	1.00	19.98
ATOM	965	C	GLN	A	139	9.846	14.749	7.471	1.00	22.85
ATOM	966	O	GLN	A	139	8.791	15.282	7.528	1.00	26.66
ATOM	967	CB	GLN	A	139	9.517	12.969	5.899	1.00	18.37
ATOM	968	CG	GLN	A	139	9.684	12.643	4.450	1.00	22.02
ATOM	969	CD	GLN	A	139	10.984	11.983	4.110	1.00	22.69
ATOM	970	OE1	GLN	A	139	10.674	10.980	3.477	1.00	35.62
ATOM	971	NE2	GLN	A	139	12.195	12.405	4.410	1.00	31.70
ATOM	972	N	ASN	A	140	10.454	14.072	8.427	1.00	26.14
ATOM	973	CA	ASN	A	140	10.215	14.183	9.848	1.00	19.06
ATOM	974	C	ASN	A	140	10.941	15.429	10.293	1.00	16.99
ATOM	975	O	ASN	A	140	11.040	15.654	11.454	1.00	18.05
ATOM	976	CB	ASN	A	140	10.581	12.910	10.541	1.00	17.20
ATOM	977	CG	ASN	A	140	9.465	11.998	10.210	1.00	16.28
ATOM	978	OD1	ASN	A	140	8.615	12.565	9.563	1.00	23.57
ATOM	979	ND2	ASN	A	140	9.460	10.756	10.630	1.00	22.65
ATOM	980	N	ARG	A	141	11.457	16.162	9.397	1.00	19.20
ATOM	981	CA	ARG	A	141	12.170	17.350	9.790	1.00	26.25
ATOM	982	C	ARG	A	141	13.219	17.090	10.818	1.00	25.06
ATOM	983	O	ARG	A	141	13.365	17.928	11.649	1.00	27.60
ATOM	984	CB	ARG	A	141	11.123	18.299	10.271	1.00	37.72
ATOM	985	CG	ARG	A	141	10.083	18.974	9.372	1.00	49.61
ATOM	986	N	GLY	A	142	14.110	16.165	10.920	1.00	19.42
ATOM	987	CA	GLY	A	142	14.997	15.778	11.902	1.00	14.21
ATOM	988	C	GLY	A	142	14.652	15.066	13.158	1.00	19.42
ATOM	989	O	GLY	A	142	15.547	14.759	13.971	1.00	23.74
ATOM	990	N	GLY	A	143	13.354	14.851	13.569	1.00	14.09
ATOM	991	CA	GLY	A	143	13.210	14.075	14.757	1.00	11.80
ATOM	992	C	GLY	A	143	12.203	12.972	14.555	1.00	16.69
ATOM	993	O	GLY	A	143	11.760	12.787	13.481	1.00	19.57
ATOM	994	N	ILE	A	144	11.668	12.386	15.590	1.00	19.71
ATOM	995	CA	ILE	A	144	10.494	11.589	15.667	1.00	20.13
ATOM	996	C	ILE	A	144	9.313	12.315	16.296	1.00	27.00
ATOM	997	O	ILE	A	144	9.298	13.026	17.268	1.00	26.75
ATOM	998	CB	ILE	A	144	10.973	10.583	16.692	1.00	16.84
ATOM	999	CG1	ILE	A	144	12.363	9.956	16.348	1.00	5.60
ATOM	1000	CG2	ILE	A	144	9.882	9.636	16.775	1.00	14.01
ATOM	1001	CD1	ILE	A	144	12.437	9.156	17.562	1.00	2.75
ATOM	1002	N	PRO	A	145	8.249	12.380	15.499	1.00	32.77
ATOM	1003	CA	PRO	A	145	6.959	12.993	15.779	1.00	29.89
ATOM	1004	C	PRO	A	145	6.484	12.588	17.180	1.00	27.78
ATOM	1005	O	PRO	A	145	6.475	11.446	17.537	1.00	26.07
ATOM	1006	CB	PRO	A	145	5.957	12.384	14.784	1.00	26.51
ATOM	1007	CG	PRO	A	145	6.887	12.059	13.668	1.00	25.85

ATOM	1008	CD	PRO	A	145	8.174	11.563	14.234	1.00	31.33
ATOM	1009	N	ASN	A	146	5.796	13.462	17.878	1.00	27.07
ATOM	1010	CA	ASN	A	146	5.454	13.274	19.230	1.00	28.59
ATOM	1011	C	ASN	A	146	6.526	12.605	20.045	1.00	29.25
ATOM	1012	O	ASN	A	146	6.087	11.995	20.996	1.00	35.51
ATOM	1013	CB	ASN	A	146	4.285	12.364	19.230	1.00	41.13
ATOM	1014	CG	ASN	A	146	3.300	12.568	18.120	1.00	48.43
ATOM	1015	OD1	ASN	A	146	3.134	13.721	17.788	1.00	49.24
ATOM	1016	ND2	ASN	A	146	2.763	11.437	17.695	1.00	47.79
ATOM	1017	N	TYR	A	147	7.791	12.799	19.885	1.00	23.88
ATOM	1018	CA	TYR	A	147	8.689	12.339	20.969	1.00	21.90
ATOM	1019	C	TYR	A	147	9.583	13.495	21.285	1.00	22.57
ATOM	1020	O	TYR	A	147	9.777	14.399	20.494	1.00	26.53
ATOM	1021	CB	TYR	A	147	9.309	11.098	20.498	1.00	21.16
ATOM	1022	CG	TYR	A	147	10.285	10.471	21.349	1.00	20.45
ATOM	1023	CD1	TYR	A	147	9.882	9.720	22.384	1.00	24.28
ATOM	1024	CD2	TYR	A	147	11.608	10.564	21.189	1.00	17.96
ATOM	1025	CE1	TYR	A	147	10.681	9.029	23.273	1.00	24.55
ATOM	1026	CE2	TYR	A	147	12.509	9.948	21.983	1.00	20.73
ATOM	1027	CZ	TYR	A	147	12.022	9.184	23.030	1.00	24.61
ATOM	1028	OH	TYR	A	147	12.891	8.536	23.887	1.00	24.80
ATOM	1029	N	PRO	A	148	9.893	13.858	22.507	1.00	22.86
ATOM	1030	CA	PRO	A	148	10.817	14.916	22.769	1.00	21.77
ATOM	1031	C	PRO	A	148	12.127	14.882	21.957	1.00	22.49
ATOM	1032	O	PRO	A	148	13.007	14.004	22.117	1.00	22.31
ATOM	1033	CB	PRO	A	148	11.185	14.694	24.251	1.00	23.23
ATOM	1034	CG	PRO	A	148	10.324	13.576	24.719	1.00	23.39
ATOM	1035	CD	PRO	A	148	9.677	12.889	23.590	1.00	25.33
ATOM	1036	N	ARG	A	149	12.432	15.980	21.250	1.00	25.45
ATOM	1037	CA	ARG	A	149	13.735	16.138	20.567	1.00	22.54
ATOM	1038	C	ARG	A	149	14.910	16.018	21.499	1.00	21.28
ATOM	1039	O	ARG	A	149	15.860	15.477	21.015	1.00	16.61
ATOM	1040	CB	ARG	A	149	13.829	17.346	19.727	1.00	31.02
ATOM	1041	CG	ARG	A	149	12.837	17.750	18.719	1.00	58.26
ATOM	1042	CD	ARG	A	149	13.452	18.605	17.658	1.00	80.58
ATOM	1043	NE	ARG	A	149	13.769	17.798	16.491	1.00	92.05
ATOM	1044	CZ	ARG	A	149	13.315	18.154	15.320	1.00	91.85
ATOM	1045	NH1	ARG	A	149	12.586	19.213	15.165	1.00	86.98
ATOM	1046	NH2	ARG	A	149	13.544	17.488	14.242	1.00	91.61
ATOM	1047	N	GLU	A	150	14.813	16.282	22.825	1.00	28.09
ATOM	1048	CA	GLU	A	150	15.950	16.171	23.735	1.00	25.55
ATOM	1049	C	GLU	A	150	16.272	14.736	24.020	1.00	21.12
ATOM	1050	O	GLU	A	150	17.372	14.443	24.371	1.00	24.39
ATOM	1051	CB	GLU	A	150	15.753	17.040	24.917	1.00	38.73
ATOM	1052	CG	GLU	A	150	14.328	17.370	25.359	1.00	67.27
ATOM	1053	CD	GLU	A	150	14.252	17.185	26.899	1.00	85.05
ATOM	1054	OE1	GLU	A	150	15.005	17.890	27.657	1.00	90.70
ATOM	1055	OE2	GLU	A	150	13.454	16.321	27.373	1.00	91.68

ATOM	1056	N	ARG	A	151	15.396	13.807	23.727	1.00	19.70
ATOM	1057	CA	ARG	A	151	15.752	12.424	23.844	1.00	19.52
ATOM	1058	C	ARG	A	151	16.163	11.779	22.531	1.00	19.28
ATOM	1059	O	ARG	A	151	16.373	10.586	22.480	1.00	14.55
ATOM	1060	CB	ARG	A	151	14.548	11.796	24.412	1.00	23.06
ATOM	1061	CG	ARG	A	151	13.853	12.432	25.516	1.00	22.24
ATOM	1062	CD	ARG	A	151	13.200	11.451	26.393	1.00	33.40
ATOM	1063	NE	ARG	A	151	12.609	11.893	27.633	1.00	46.53
ATOM	1064	CZ	ARG	A	151	11.796	11.028	28.275	1.00	52.87
ATOM	1065	NH1	ARG	A	151	11.428	9.823	27.930	1.00	51.02
ATOM	1066	NH2	ARG	A	151	11.203	11.278	29.416	1.00	59.98
ATOM	1067	N	THR	A	152	16.360	12.526	21.505	1.00	14.12
ATOM	1068	CA	THR	A	152	16.629	11.925	20.253	1.00	15.05
ATOM	1069	C	THR	A	152	17.995	12.249	19.745	1.00	17.30
ATOM	1070	O	THR	A	152	18.282	13.373	19.965	1.00	21.34
ATOM	1071	CB	THR	A	152	15.680	12.408	19.158	1.00	13.91
ATOM	1072	OG1	THR	A	152	14.423	12.256	19.858	1.00	23.92
ATOM	1073	CG2	THR	A	152	15.737	11.934	17.759	1.00	6.77
ATOM	1074	N	LYS	A	153	18.704	11.336	19.121	1.00	15.49
ATOM	1075	CA	LYS	A	153	19.930	11.725	18.450	1.00	17.73
ATOM	1076	C	LYS	A	153	19.893	11.035	17.073	1.00	18.41
ATOM	1077	O	LYS	A	153	19.866	9.800	17.121	1.00	16.04
ATOM	1078	CB	LYS	A	153	21.112	11.260	19.338	1.00	14.55
ATOM	1079	CG	LYS	A	153	22.523	11.508	18.933	1.00	11.95
ATOM	1080	CD	LYS	A	153	22.883	12.882	19.403	1.00	40.35
ATOM	1081	CE	LYS	A	153	24.358	13.093	19.079	1.00	62.12
ATOM	1082	NZ	LYS	A	153	24.930	14.235	19.863	1.00	73.03
ATOM	1083	N	VAL	A	154	19.910	11.962	16.136	1.00	15.86
ATOM	1084	CA	VAL	A	154	20.031	11.508	14.730	1.00	15.79
ATOM	1085	C	VAL	A	154	21.406	11.481	14.040	1.00	13.11
ATOM	1086	O	VAL	A	154	21.958	12.460	13.675	1.00	13.51
ATOM	1087	CB	VAL	A	154	19.095	12.257	13.674	1.00	5.90
ATOM	1088	CG1	VAL	A	154	19.276	11.765	12.247	1.00	8.45
ATOM	1089	CG2	VAL	A	154	17.672	12.091	14.117	1.00	7.14
ATOM	1090	N	PHE	A	155	22.039	10.448	13.605	1.00	13.75
ATOM	1091	CA	PHE	A	155	23.263	10.473	12.843	1.00	10.67
ATOM	1092	C	PHE	A	155	22.906	10.406	11.402	1.00	11.64
ATOM	1093	O	PHE	A	155	22.505	9.367	10.893	1.00	15.09
ATOM	1094	CB	PHE	A	155	23.955	9.120	13.304	1.00	5.38
ATOM	1095	CG	PHE	A	155	24.396	9.266	14.739	1.00	16.52
ATOM	1096	CD1	PHE	A	155	23.678	8.642	15.696	1.00	23.70
ATOM	1097	CD2	PHE	A	155	25.503	9.950	15.107	1.00	11.27
ATOM	1098	CE1	PHE	A	155	24.037	8.702	17.011	1.00	23.25
ATOM	1099	CE2	PHE	A	155	25.888	9.994	16.372	1.00	7.37
ATOM	1100	CZ	PHE	A	155	25.139	9.384	17.357	1.00	16.13
ATOM	1101	N	CYS	A	156	23.205	11.255	10.511	1.00	12.38
ATOM	1102	CA	CYS	A	156	22.847	11.443	9.114	1.00	11.64
ATOM	1103	C	CYS	A	156	24.057	12.027	8.461	1.00	10.08

ATOM	1104	O	CYS	A	156	24.385	13.174	8.378	1.00	13.73
ATOM	1105	CB	CYS	A	156	21.575	12.391	8.917	1.00	6.30
ATOM	1106	SG	CYS	A	156	20.137	11.470	8.287	1.00	10.60
ATOM	1107	N	ASN	A	157	24.814	11.147	7.918	1.00	16.95
ATOM	1108	CA	ASN	A	157	26.229	11.665	7.576	1.00	19.16
ATOM	1109	C	ASN	A	157	26.197	12.367	6.310	1.00	17.70
ATOM	1110	O	ASN	A	157	25.368	12.330	5.469	1.00	20.91
ATOM	1111	CB	ASN	A	157	27.115	10.714	8.300	1.00	30.34
ATOM	1112	CG	ASN	A	157	27.733	9.498	7.932	1.00	34.95
ATOM	1113	OD1	ASN	A	157	28.011	8.573	8.606	1.00	44.28
ATOM	1114	ND2	ASN	A	157	27.965	9.541	6.660	1.00	54.18
ATOM	1115	N	VAL	A	158	26.849	13.501	6.313	1.00	25.65
ATOM	1116	CA	VAL	A	158	26.825	14.483	5.192	1.00	28.21
ATOM	1117	C	VAL	A	158	26.768	13.893	3.758	1.00	24.85
ATOM	1118	O	VAL	A	158	25.732	14.266	3.111	1.00	30.96
ATOM	1119	CB	VAL	A	158	27.954	15.512	5.217	1.00	27.87
ATOM	1120	CG1	VAL	A	158	28.751	14.595	4.238	1.00	40.51
ATOM	1121	CG2	VAL	A	158	27.791	16.704	4.399	1.00	34.39
ATOM	1122	N	GLY	A	159	27.483	12.956	3.016	1.00	5.94
ATOM	1123	CA	GLY	A	159	26.713	12.774	1.732	1.00	6.20
ATOM	1124	C	GLY	A	159	25.734	11.797	1.487	1.00	4.00
ATOM	1125	O	GLY	A	159	25.732	10.704	0.848	1.00	4.06
ATOM	1126	N	ASP	A	160	25.052	11.441	2.643	1.00	8.53
ATOM	1127	CA	ASP	A	160	24.106	10.302	2.828	1.00	11.97
ATOM	1128	C	ASP	A	160	22.755	10.698	2.177	1.00	14.44
ATOM	1129	O	ASP	A	160	21.928	11.398	2.692	1.00	10.21
ATOM	1130	CB	ASP	A	160	24.037	9.829	4.277	1.00	12.43
ATOM	1131	CG	ASP	A	160	23.126	8.629	4.261	1.00	20.99
ATOM	1132	OD1	ASP	A	160	22.525	8.408	3.179	1.00	33.03
ATOM	1133	OD2	ASP	A	160	22.956	7.840	5.216	1.00	10.13
ATOM	1134	N	ALA	A	161	22.455	10.402	0.961	1.00	12.33
ATOM	1135	CA	ALA	A	161	21.318	10.743	0.269	1.00	11.01
ATOM	1136	C	ALA	A	161	19.961	10.317	0.848	1.00	15.22
ATOM	1137	O	ALA	A	161	18.969	11.034	0.594	1.00	9.50
ATOM	1138	CB	ALA	A	161	21.365	10.334	-1.172	1.00	13.68
ATOM	1139	N	VAL	A	162	19.915	9.468	1.840	1.00	14.54
ATOM	1140	CA	VAL	A	162	18.653	9.014	2.287	1.00	9.86
ATOM	1141	C	VAL	A	162	18.235	10.063	3.258	1.00	13.50
ATOM	1142	O	VAL	A	162	17.094	10.458	3.377	1.00	20.47
ATOM	1143	CB	VAL	A	162	18.596	7.778	3.117	1.00	7.34
ATOM	1144	CG1	VAL	A	162	18.931	6.592	2.259	1.00	6.50
ATOM	1145	CG2	VAL	A	162	19.514	7.858	4.210	1.00	18.46
ATOM	1146	N	CYS	A	163	19.198	10.733	3.719	1.00	13.44
ATOM	1147	CA	CYS	A	163	18.864	11.811	4.720	1.00	11.26
ATOM	1148	C	CYS	A	163	18.256	12.963	4.042	1.00	15.57
ATOM	1149	O	CYS	A	163	18.219	13.857	4.880	1.00	14.09
ATOM	1150	CB	CYS	A	163	20.144	12.145	5.570	1.00	18.70
ATOM	1151	SG	CYS	A	163	20.748	10.705	6.581	1.00	13.38

ATOM	1152	N	THR	A	164	18.100	13.014	2.696	1.00	21.82
ATOM	1153	CA	THR	A	164	17.603	14.283	2.171	1.00	23.08
ATOM	1154	C	THR	A	164	16.597	14.022	1.098	1.00	23.39
ATOM	1155	O	THR	A	164	16.517	14.727	0.137	1.00	33.37
ATOM	1156	CB	THR	A	164	18.463	15.341	1.454	1.00	23.25
ATOM	1157	OG1	THR	A	164	19.486	14.707	0.674	1.00	23.21
ATOM	1158	CG2	THR	A	164	18.958	16.261	2.491	1.00	37.71
ATOM	1159	N	GLY	A	165	15.802	13.085	1.309	1.00	24.23
ATOM	1160	CA	GLY	A	165	14.606	12.783	0.579	1.00	26.69
ATOM	1161	C	GLY	A	165	14.699	11.814	-0.515	1.00	28.56
ATOM	1162	O	GLY	A	165	13.680	11.775	-1.124	1.00	39.76
ATOM	1163	N	THR	A	166	15.661	11.044	-0.736	1.00	25.80
ATOM	1164	CA	THR	A	166	16.006	10.220	-1.774	1.00	25.53
ATOM	1165	C	THR	A	166	16.195	8.866	-1.175	1.00	25.35
ATOM	1166	O	THR	A	166	16.913	8.760	-0.206	1.00	30.91
ATOM	1167	CB	THR	A	166	17.406	10.657	-2.230	1.00	31.57
ATOM	1168	OG1	THR	A	166	17.105	11.788	-2.982	1.00	24.13
ATOM	1169	CG2	THR	A	166	18.061	9.559	-2.983	1.00	34.67
ATOM	1170	N	LEU	A	167	15.734	7.833	-1.817	1.00	19.63
ATOM	1171	CA	LEU	A	167	16.219	6.552	-1.465	1.00	16.11
ATOM	1172	C	LEU	A	167	17.395	6.044	-2.300	1.00	19.87
ATOM	1173	O	LEU	A	167	17.265	4.869	-2.612	1.00	21.38
ATOM	1174	CB	LEU	A	167	15.086	5.624	-1.555	1.00	23.45
ATOM	1175	CG	LEU	A	167	14.123	5.773	-0.401	1.00	33.91
ATOM	1176	CD1	LEU	A	167	12.969	4.908	-0.793	1.00	42.10
ATOM	1177	CD2	LEU	A	167	14.776	5.385	0.903	1.00	25.86
ATOM	1178	N	ILE	A	168	18.534	6.726	-2.507	1.00	21.67
ATOM	1179	CA	ILE	A	168	19.608	6.051	-3.170	1.00	23.38
ATOM	1180	C	ILE	A	168	20.675	5.585	-2.189	1.00	20.47
ATOM	1181	O	ILE	A	168	21.139	6.541	-1.581	1.00	18.08
ATOM	1182	CB	ILE	A	168	20.254	6.835	-4.297	1.00	23.50
ATOM	1183	CG1	ILE	A	168	21.232	7.874	-3.800	1.00	13.71
ATOM	1184	CG2	ILE	A	168	19.445	7.627	-5.276	1.00	18.16
ATOM	1185	CD1	ILE	A	168	20.908	8.938	-4.804	1.00	26.95
ATOM	1186	N	ILE	A	169	21.396	4.478	-2.394	1.00	18.32
ATOM	1187	CA	ILE	A	169	22.554	4.448	-1.536	1.00	13.25
ATOM	1188	C	ILE	A	169	23.924	4.662	-1.967	1.00	11.95
ATOM	1189	O	ILE	A	169	24.615	3.942	-2.539	1.00	20.35
ATOM	1190	CB	ILE	A	169	22.503	3.351	-0.499	1.00	21.07
ATOM	1191	CG1	ILE	A	169	23.398	2.181	-0.655	1.00	11.06
ATOM	1192	CG2	ILE	A	169	21.122	2.801	-0.533	1.00	7.02
ATOM	1193	CD1	ILE	A	169	22.581	1.266	-1.587	1.00	32.83
ATOM	1194	N	THR	A	170	24.570	5.586	-1.296	1.00	17.16
ATOM	1195	CA	THR	A	170	25.883	6.217	-1.397	1.00	13.01
ATOM	1196	C	THR	A	170	26.722	5.719	-0.240	1.00	10.14
ATOM	1197	O	THR	A	170	26.334	5.036	0.758	1.00	9.98
ATOM	1198	CB	THR	A	170	25.623	7.713	-1.344	1.00	15.02
ATOM	1199	OG1	THR	A	170	26.466	7.947	-0.255	1.00	23.39

ATOM	1200	CG2	THR	A	170	24.389	7.914	-0.452	1.00	41.10
ATOM	1201	N	PRO	A	171	28.000	5.738	-0.469	1.00	10.12
ATOM	1202	CA	PRO	A	171	29.012	5.066	0.339	1.00	11.88
ATOM	1203	C	PRO	A	171	28.897	5.492	1.765	1.00	9.74
ATOM	1204	O	PRO	A	171	28.904	4.682	2.646	1.00	9.54
ATOM	1205	CB	PRO	A	171	30.414	5.207	-0.286	1.00	7.15
ATOM	1206	CG	PRO	A	171	30.017	5.603	-1.654	1.00	7.18
ATOM	1207	CD	PRO	A	171	28.667	6.233	-1.601	1.00	6.90
ATOM	1208	N	ALA	A	172	28.725	6.718	1.980	1.00	6.71
ATOM	1209	CA	ALA	A	172	28.247	7.315	3.169	1.00	8.62
ATOM	1210	C	ALA	A	172	27.075	6.631	3.892	1.00	10.99
ATOM	1211	O	ALA	A	172	27.037	6.755	5.165	1.00	16.49
ATOM	1212	CB	ALA	A	172	27.904	8.812	3.040	1.00	2.86
ATOM	1213	N	HIS	A	173	26.287	5.815	3.278	1.00	6.36
ATOM	1214	CA	HIS	A	173	25.133	5.468	4.081	1.00	5.29
ATOM	1215	C	HIS	A	173	25.685	4.314	4.888	1.00	10.58
ATOM	1216	O	HIS	A	173	25.082	3.598	5.668	1.00	9.36
ATOM	1217	CB	HIS	A	173	24.081	4.883	3.216	1.00	8.41
ATOM	1218	CG	HIS	A	173	22.815	4.403	3.791	1.00	7.30
ATOM	1219	ND1	HIS	A	173	22.066	5.327	4.565	1.00	8.48
ATOM	1220	CD2	HIS	A	173	22.148	3.264	3.670	1.00	7.83
ATOM	1221	CE1	HIS	A	173	20.932	4.657	4.861	1.00	17.36
ATOM	1222	NE2	HIS	A	173	20.945	3.423	4.379	1.00	5.29
ATOM	1223	N	LEU	A	174	26.823	3.947	4.326	1.00	8.03
ATOM	1224	CA	LEU	A	174	27.344	2.623	4.682	1.00	8.06
ATOM	1225	C	LEU	A	174	28.171	2.787	5.930	1.00	13.06
ATOM	1226	O	LEU	A	174	28.609	1.648	6.151	1.00	19.88
ATOM	1227	CB	LEU	A	174	28.078	2.118	3.488	1.00	2.76
ATOM	1228	CG	LEU	A	174	27.560	0.902	2.847	1.00	13.35
ATOM	1229	CD1	LEU	A	174	26.024	1.017	2.796	1.00	18.01
ATOM	1230	CD2	LEU	A	174	27.913	0.740	1.421	1.00	21.70
ATOM	1231	N	SER	A	175	28.290	3.989	6.447	1.00	12.43
ATOM	1232	CA	SER	A	175	29.230	4.052	7.553	1.00	18.01
ATOM	1233	C	SER	A	175	28.872	4.811	8.847	1.00	19.89
ATOM	1234	O	SER	A	175	28.968	6.047	9.120	1.00	14.61
ATOM	1235	CB	SER	A	175	30.516	4.606	6.847	1.00	20.11
ATOM	1236	OG	SER	A	175	30.834	5.907	7.293	1.00	27.73
ATOM	1237	N	TYR	A	176	28.479	3.978	9.815	1.00	17.89
ATOM	1238	CA	TYR	A	176	28.092	4.530	11.133	1.00	12.54
ATOM	1239	C	TYR	A	176	28.530	3.671	12.272	1.00	11.16
ATOM	1240	O	TYR	A	176	27.949	3.770	13.257	1.00	7.63
ATOM	1241	CB	TYR	A	176	26.511	4.283	11.053	1.00	9.13
ATOM	1242	CG	TYR	A	176	25.831	5.525	10.029	1.00	5.03
ATOM	1243	CD1	TYR	A	176	25.874	6.923	10.425	1.00	2.75
ATOM	1244	CD2	TYR	A	176	25.152	5.022	8.980	1.00	2.18
ATOM	1245	CE1	TYR	A	176	25.287	7.754	9.633	1.00	4.25
ATOM	1246	CE2	TYR	A	176	24.649	5.981	8.085	1.00	6.77
ATOM	1247	CZ	TYR	A	176	24.658	7.329	8.399	1.00	6.22

ATOM	1248	OH	TYR	A	176	24.074	8.375	7.635	1.00	5.76
ATOM	1249	N	THR	A	177	29.430	2.685	12.167	1.00	10.72
ATOM	1250	CA	THR	A	177	29.797	1.854	13.284	1.00	13.31
ATOM	1251	C	THR	A	177	30.516	2.659	14.320	1.00	12.46
ATOM	1252	O	THR	A	177	30.311	2.436	15.475	1.00	13.12
ATOM	1253	CB	THR	A	177	30.658	0.683	12.798	1.00	3.49
ATOM	1254	OG1	THR	A	177	31.361	1.247	11.870	1.00	32.08
ATOM	1255	CG2	THR	A	177	29.675	-0.149	12.083	1.00	6.42
ATOM	1256	N	ILE	A	178	31.409	3.474	13.920	1.00	10.48
ATOM	1257	CA	ILE	A	178	32.203	4.246	14.783	1.00	15.25
ATOM	1258	C	ILE	A	178	31.180	5.045	15.632	1.00	16.95
ATOM	1259	O	ILE	A	178	31.092	4.774	16.851	1.00	22.68
ATOM	1260	CB	ILE	A	178	33.338	5.121	14.357	1.00	25.11
ATOM	1261	CG1	ILE	A	178	34.701	4.496	14.056	1.00	25.05
ATOM	1262	CG2	ILE	A	178	33.599	6.205	15.392	1.00	27.60
ATOM	1263	CD1	ILE	A	178	34.553	3.006	14.071	1.00	55.86
ATOM	1264	N	GLU	A	179	30.218	5.799	15.178	1.00	16.34
ATOM	1265	CA	GLU	A	179	29.290	6.610	15.985	1.00	16.94
ATOM	1266	C	GLU	A	179	28.324	5.713	16.692	1.00	14.79
ATOM	1267	O	GLU	A	179	27.683	6.012	17.716	1.00	19.20
ATOM	1268	CB	GLU	A	179	28.555	7.637	15.169	1.00	21.16
ATOM	1269	CG	GLU	A	179	28.790	7.283	13.691	1.00	50.37
ATOM	1270	CD	GLU	A	179	29.933	7.701	12.851	1.00	61.82
ATOM	1271	OE1	GLU	A	179	30.163	8.890	12.697	1.00	77.56
ATOM	1272	OE2	GLU	A	179	30.627	6.854	12.309	1.00	75.83
ATOM	1273	N	ALA	A	180	28.240	4.418	16.412	1.00	8.00
ATOM	1274	CA	ALA	A	180	27.353	3.520	17.042	1.00	14.34
ATOM	1275	C	ALA	A	180	28.048	2.991	18.280	1.00	19.53
ATOM	1276	O	ALA	A	180	27.397	3.142	19.265	1.00	21.17
ATOM	1277	CB	ALA	A	180	26.843	2.437	16.128	1.00	11.97
ATOM	1278	N	ARG	A	181	29.317	2.547	18.287	1.00	21.89
ATOM	1279	CA	ARG	A	181	29.992	1.982	19.398	1.00	16.48
ATOM	1280	C	ARG	A	181	30.296	3.106	20.367	1.00	19.44
ATOM	1281	O	ARG	A	181	30.243	3.104	21.639	1.00	28.53
ATOM	1282	CB	ARG	A	181	31.310	1.408	19.143	1.00	12.43
ATOM	1283	CG	ARG	A	181	31.954	0.432	20.052	1.00	45.44
ATOM	1284	CD	ARG	A	181	32.596	-0.688	19.242	1.00	66.21
ATOM	1285	NE	ARG	A	181	33.333	-0.030	18.164	1.00	85.83
ATOM	1286	CZ	ARG	A	181	33.306	-0.321	16.895	1.00	91.35
ATOM	1287	NH1	ARG	A	181	32.551	-1.320	16.530	1.00	96.98
ATOM	1288	NH2	ARG	A	181	34.023	0.400	16.095	1.00	92.83
ATOM	1289	N	GLY	A	182	30.387	4.262	19.847	1.00	13.94
ATOM	1290	CA	GLY	A	182	30.553	5.404	20.728	1.00	7.40
ATOM	1291	C	GLY	A	182	29.741	6.574	20.960	1.00	7.95
ATOM	1292	O	GLY	A	182	29.171	6.512	22.083	1.00	12.73
ATOM	1293	N	GLU	A	183	29.725	7.622	20.138	1.00	6.42
ATOM	1294	CA	GLU	A	183	28.816	8.775	20.405	1.00	10.04
ATOM	1295	C	GLU	A	183	27.421	8.369	20.645	1.00	14.41

ATOM	1296	O	GLU	A	183	26.846	8.530	21.749	1.00	15.43
ATOM	1297	CB	GLU	A	183	29.053	9.791	19.402	1.00	21.24
ATOM	1298	CG	GLU	A	183	28.079	10.638	18.725	1.00	62.21
ATOM	1299	CD	GLU	A	183	28.248	12.103	19.141	1.00	81.34
ATOM	1300	OE1	GLU	A	183	28.850	12.243	20.232	1.00	95.85
ATOM	1301	OE2	GLU	A	183	27.791	13.027	18.430	1.00	90.85
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ATOM	1303	CA	ALA	A	184	25.444	7.083	20.117	1.00	14.54
ATOM	1304	C	ALA	A	184	25.549	6.382	21.464	1.00	13.62
ATOM	1305	O	ALA	A	184	24.575	6.533	22.215	1.00	16.75
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ATOM	1307	N	ALA	A	185	26.428	5.396	21.774	1.00	9.42
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ATOM	1312	N	ARG	A	186	27.271	6.673	24.090	1.00	15.54
ATOM	1313	CA	ARG	A	186	27.352	7.507	25.300	1.00	13.57
ATOM	1314	C	ARG	A	186	26.085	8.286	25.561	1.00	11.49
ATOM	1315	O	ARG	A	186	25.421	8.267	26.573	1.00	8.74
ATOM	1316	CB	ARG	A	186	28.484	8.460	25.043	1.00	30.29
ATOM	1317	CG	ARG	A	186	29.869	7.851	25.240	1.00	37.15
ATOM	1318	CD	ARG	A	186	30.983	8.826	24.813	1.00	42.36
ATOM	1319	NE	ARG	A	186	31.902	7.942	24.064	1.00	51.82
ATOM	1320	CZ	ARG	A	186	32.324	8.346	22.870	1.00	50.20
ATOM	1321	NH1	ARG	A	186	31.924	9.538	22.424	1.00	47.65
ATOM	1322	NH2	ARG	A	186	33.115	7.476	22.318	1.00	39.90
ATOM	1323	N	PHE	A	187	25.565	8.774	24.434	1.00	8.37
ATOM	1324	CA	PHE	A	187	24.195	9.370	24.426	1.00	13.48
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ATOM	1326	O	PHE	A	187	22.379	8.916	25.995	1.00	14.81
ATOM	1327	CB	PHE	A	187	23.667	9.791	23.087	1.00	11.81
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ATOM	1332	CE2	PHE	A	187	19.895	10.026	22.485	1.00	17.42
ATOM	1333	CZ	PHE	A	187	19.661	11.322	22.924	1.00	3.70
ATOM	1334	N	LEU	A	188	23.033	7.232	24.803	1.00	15.17
ATOM	1335	CA	LEU	A	188	21.908	6.427	25.324	1.00	18.43
ATOM	1336	C	LEU	A	188	22.207	6.221	26.775	1.00	19.67
ATOM	1337	O	LEU	A	188	21.280	6.512	27.461	1.00	18.17
ATOM	1338	CB	LEU	A	188	21.703	5.088	24.552	1.00	18.72
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ATOM	1343	CA	ARG	A	189	23.798	5.812	28.547	1.00	18.41

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ATOM   1344  C   ARG A 189      23.353   7.039  29.321  1.00 16.87
ATOM   1345  O   ARG A 189      22.852   7.164  30.389  1.00 13.64
ATOM   1346  CB  ARG A 189      25.325   6.017  28.529  1.00 21.93
ATOM   1347  CG  ARG A 189      25.882   5.624  29.894  1.00 19.95
ATOM   1348  CD  ARG A 189      27.239   6.140  30.235  1.00 21.42
ATOM   1349  NE  ARG A 189      27.257   7.545  29.926  1.00 25.62
ATOM   1350  CZ  ARG A 189      28.491   7.983  29.699  1.00 29.22
ATOM   1351  NH1 ARG A 189      29.315   6.960  29.840  1.00 26.71
ATOM   1352  NH2 ARG A 189      28.780   9.210  29.383  1.00 33.27
ATOM   1353  N   ASP A 190      23.837   8.150  28.796  1.00 13.76
ATOM   1354  CA  ASP A 190      23.489   9.338  29.615  1.00 17.78
ATOM   1355  C   ASP A 190      22.008   9.364  29.711  1.00 16.79
ATOM   1356  O   ASP A 190      21.661   9.891  30.692  1.00 23.13
ATOM   1357  CB  ASP A 190      23.995  10.663  29.070  1.00 23.17
ATOM   1358  CG  ASP A 190      25.553  10.664  29.079  1.00 33.40
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ATOM   1360  OD2 ASP A 190      25.961  11.595  28.321  1.00 30.24
ATOM   1361  N   ARG A 191      21.156   9.128  28.781  1.00 21.61
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ATOM   1363  C   ARG A 191      19.176   8.237  29.825  1.00 21.23
ATOM   1364  O   ARG A 191      18.327   8.515  30.651  1.00 20.98
ATOM   1365  CB  ARG A 191      19.014   9.214  27.450  1.00 19.76
ATOM   1366  CG  ARG A 191      19.605  10.282  26.521  1.00 27.49
ATOM   1367  CD  ARG A 191      18.848  11.594  26.689  1.00 36.68
ATOM   1368  NE  ARG A 191      17.559  11.023  27.144  1.00 60.89
ATOM   1369  CZ  ARG A 191      16.841  11.651  28.087  1.00 73.30
ATOM   1370  NH1 ARG A 191      17.404  12.780  28.496  1.00 76.65
ATOM   1371  NH2 ARG A 191      15.675  11.224  28.574  1.00 62.02
ATOM   1372  N   ILE A 192      19.734   7.037  29.885  1.00 21.02
ATOM   1373  CA  ILE A 192      19.500   6.080  30.913  1.00 21.92
ATOM   1374  C   ILE A 192      19.705   6.598  32.337  1.00 25.67
ATOM   1375  O   ILE A 192      19.145   6.053  33.263  1.00 27.95
ATOM   1376  CB  ILE A 192      20.289   4.775  30.750  1.00 24.23
ATOM   1377  CG1 ILE A 192      19.770   4.215  29.475  1.00 26.91
ATOM   1378  CG2 ILE A 192      19.923   3.983  31.951  1.00 15.15
ATOM   1379  CD1 ILE A 192      20.418   2.954  29.019  1.00 21.07
ATOM   1380  N   ARG A 193      20.535   7.574  32.629  1.00 28.72
ATOM   1381  CA  ARG A 193      20.800   8.068  33.963  1.00 33.95
ATOM   1382  C   ARG A 193      20.116   9.377  34.406  1.00 42.87
ATOM   1383  O   ARG A 193      20.479   9.267  35.618  1.00 48.19
ATOM   1384  CB  ARG A 193      22.298   8.179  34.167  1.00 34.19
ATOM   1385  CG  ARG A 193      23.096   6.896  34.100  1.00 39.38
ATOM   1386  CD  ARG A 193      24.590   7.213  34.133  1.00 65.92
ATOM   1387  NE  ARG A 193      25.339   5.973  34.003  1.00 81.05
ATOM   1388  CZ  ARG A 193      26.631   5.765  33.770  1.00 81.52
ATOM   1389  NH1 ARG A 193      27.441   6.816  33.647  1.00 80.92
ATOM   1390  NH2 ARG A 193      27.120   4.536  33.652  1.00 74.00
ATOM   1391  OT  ARG A 193      19.292  10.277  34.082  1.00 38.80
TER
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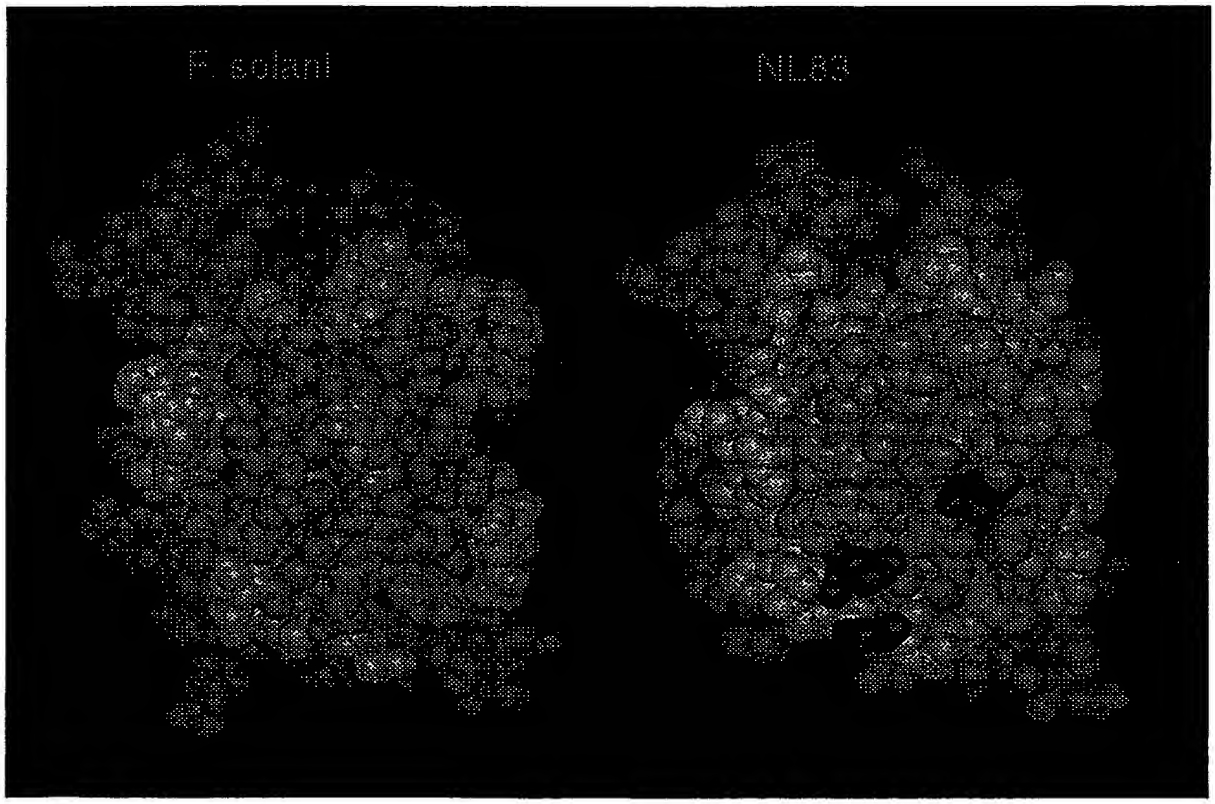

Fig. 2

3D structure of cutinases from *F. solani pisi* (left) and *H. insolens* (right)

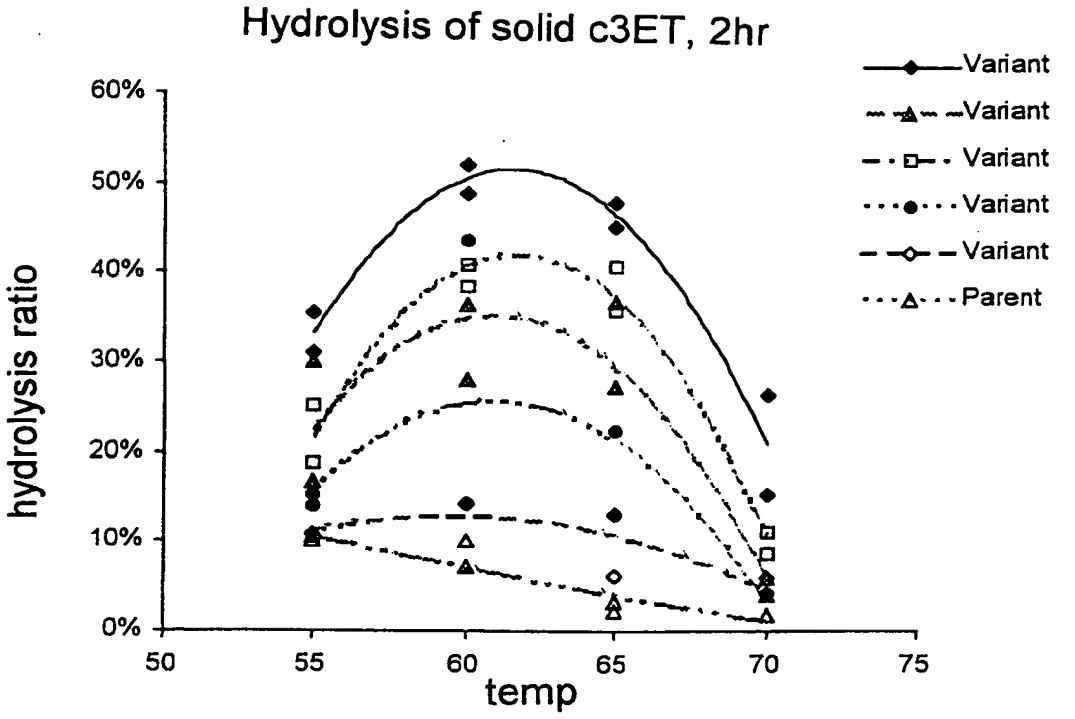

Fig. 3

Hydrolysis of solid c3ET, 2 hr

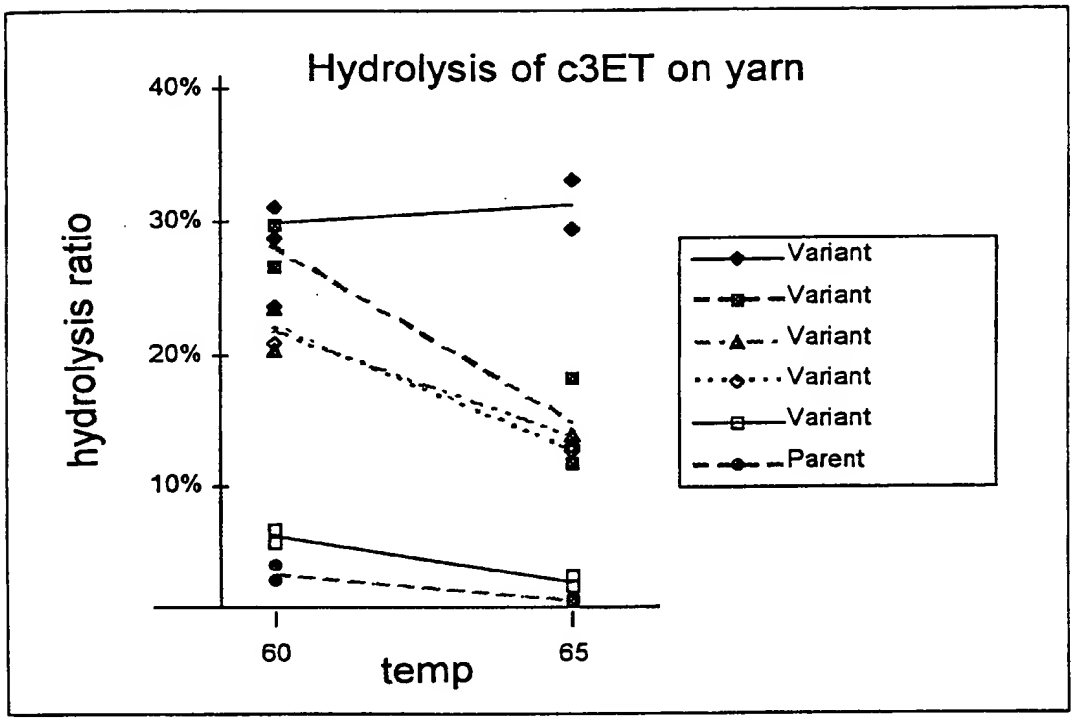

Fig. 4

Hydrolysis of c3ET on yarn, 17 hr

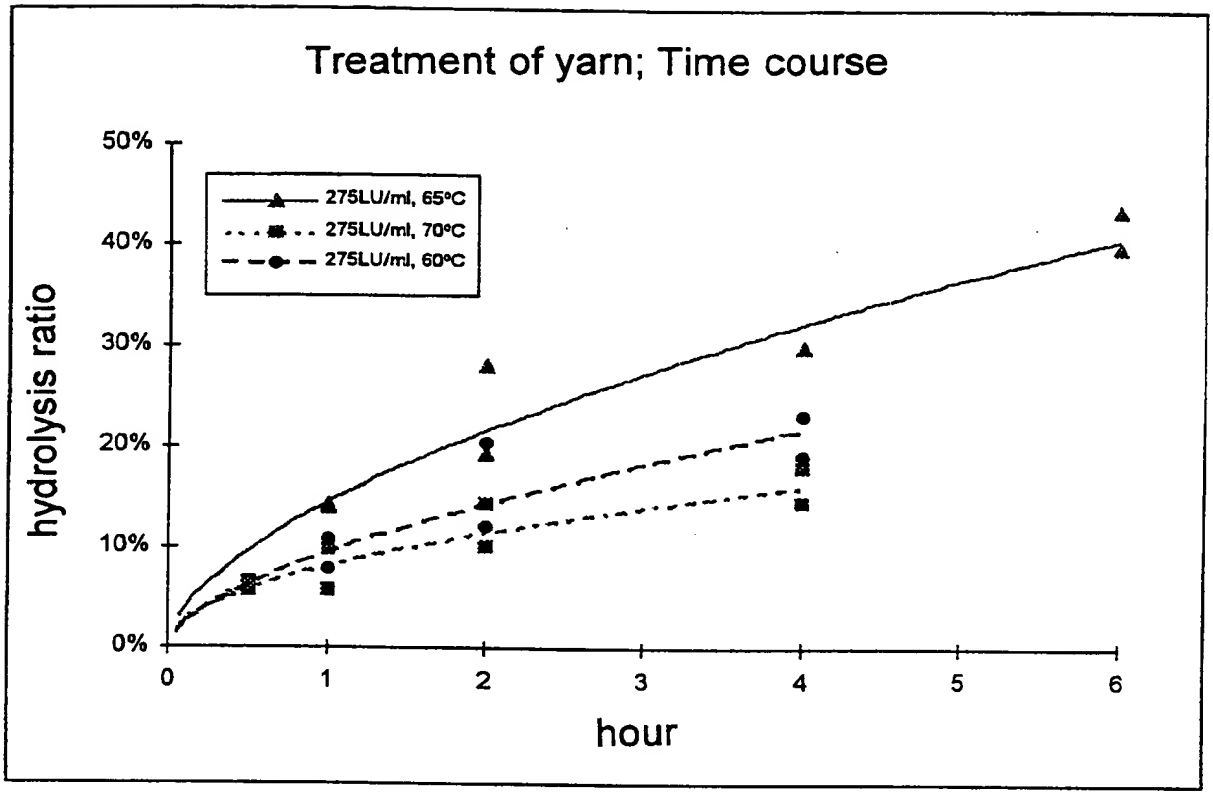

Fig. 5

Treatment of yarn; time course

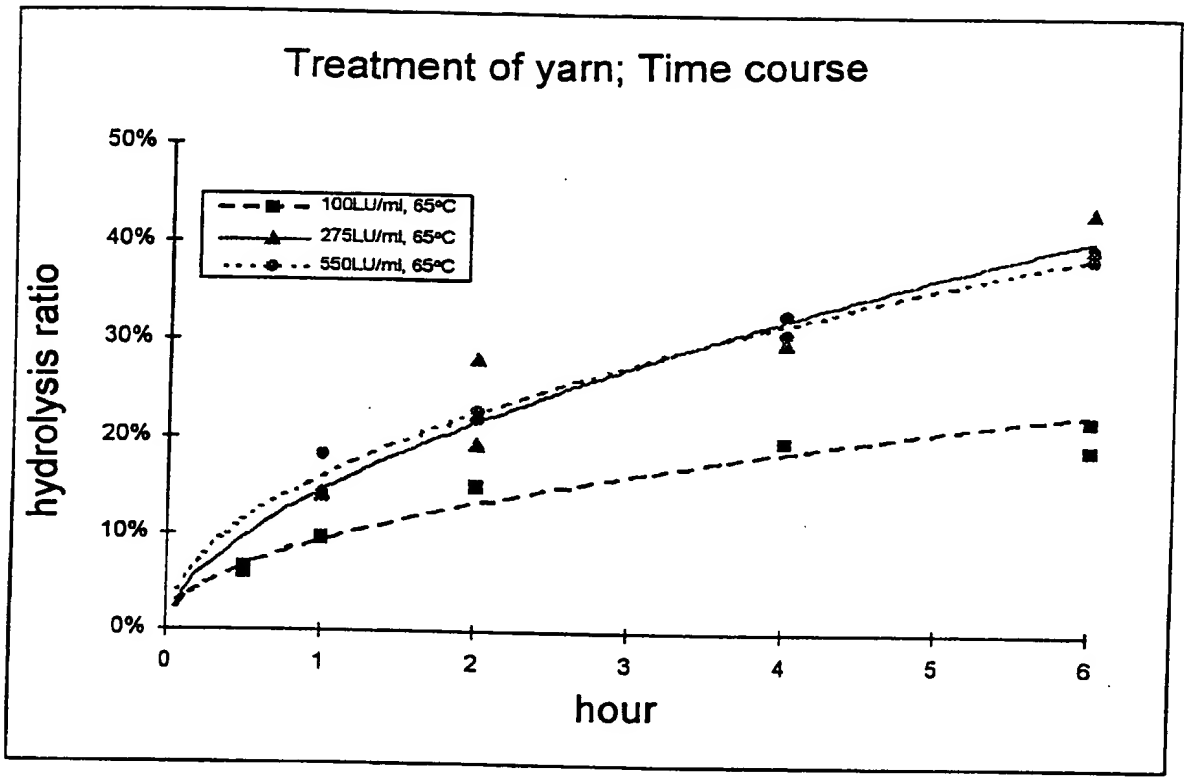

Fig. 6

Treatment of yarn; time course

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 99/00678

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C12N 9/18 // C11D 3/386, C08G 63/91
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9009446 A1 (PLANT GENETICS SYSTEMS, N.V.), 23 August 1990 (23.08.90), see page 1, lines 11-20, claims --	1-32,34
X	WO 9414963 A1 (UNILEVER N.V.), 7 July 1994 (07.07.94), see claim 14 --	1-32,34
A	WO 9414964 A1 (UNILEVER N.V.), 7 July 1994 (07.07.94) --	1-32,34
A	WO 9704078 A1 (NOVO NORDISK A/S), 6 February 1997 (06.02.97), see claim 51 --	1-32,34

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 May 2000	11 -05- 2000
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Yvonne Siösteen/EÖ Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 99/00678

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PROTEINS: Structure, Function, and Genetics, Volume 26, 1996, Sonia Longhi et al, "Dynamics of Fusarium solani Cutinase Investigated Through Structural Comparison Among Different Crystal Forms of Its Variants" page 442 - page 458 -- --------	1-32,34

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 99/00678

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).:

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see next sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-32 and 34

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 99/00678

The invention claimed relates to two different inventions :

I. Claims 1-32 and 34 relate to cutinase variants and the use of these variants.
II. Claim 33 relates to a method for detecting cutinase activity in a sample.

Unity of invention exists only when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding "special technical feature" _ i.e. features that define a contribution which each of the inventions make over prior art. (See Annex B to administrative instructions and Rule 13.1).